

Affinity Chromatography of RecA Protein and RecA Nucleoprotein Complexes on RecA Protein-Agarose Columns*

(Received for publication, December 3, 1987)

Nancy Freitag† and Kevin McEntee§

From the Laboratory of Biomedical and Environmental Sciences and the Department of Biological Chemistry, School of Medicine, University of California, Los Angeles, California 90024

We have analyzed the nature of RecA protein-RecA protein interactions using an affinity column prepared by coupling RecA protein to an agarose support. When radiolabeled soluble proteins from *Escherichia coli* are applied to this column, only the labeled RecA protein from the extract was selectively retained and bound tightly to the affinity column. Efficient binding of purified ³⁵S-labeled RecA protein required Mg²⁺, and high salt did not interfere with the binding of RecA protein to the column. Complete removal of the bound enzyme from the affinity column required treatment with guanidine HCl (5 M) or urea (8 M). These and other properties suggest that hydrophobic interactions contribute significantly to RecA protein subunit recognition in solution. Using a series of truncated RecA proteins synthesized *in vitro*, we have obtained evidence that at least some of the sequences involved in protein recognition are localized within the first 90 amino-terminal residues of the protein. Based on the observation that RecA proteins from three heterologous bacteria are specifically retained on the *E. coli* RecA affinity column, it is likely that this binding domain is highly conserved and is required for interaction and association of RecA protein monomers.

Stable ternary complexes of RecA protein and single-stranded DNA were formed in the presence of the nonhydrolyzable ATP analog adenosine 5'-O-(thiotriphosphate) and applied to the affinity columns. Most of the complexes formed with M13 DNA could be eluted in high salt, whereas a substantial fraction of those formed with the oligonucleotide (dT)₂₅₋₃₀ remained bound in high salt and were quantitatively eluted with guanidine HCl (5 M). The different binding properties of these RecA protein-DNA complexes likely reflect differences in the availability of a hydrophobic surface on RecA protein when it is bound to long polynucleotides compared to short oligonucleotides.

(1, 2). Under suitable conditions of pH and Mg²⁺, the protein can oligomerize into helical filaments extending more than 1 μm (3, 4). Filament formation has been most thoroughly investigated by right-angle light scattering of RecA protein solutions. The results of these studies indicate that high concentrations of Mg²⁺ (greater than 10 mM) are required for polymerization of RecA protein, whereas the addition of different nucleotides or high salt disrupts long filaments and severely reduces scattering (5, 6). Although long, DNA-free filaments of RecA protein are probably not intermediates in RecA protein-dependent reactions (5), they serve to illustrate the prevalence of protein-protein interactions that are fundamental biochemical properties of this enzyme.

During its participation in homology-dependent DNA pairing reactions as well as in promoting the autodigestion of both LexA and phage λ repressor proteins *in vitro*, RecA protein functions in a highly ordered nucleoprotein complex containing single-stranded DNA (7, 8). Binding studies demonstrate that RecA protein displays cooperativity in its association with single- and double-stranded DNAs (2, 9); and kinetic analysis of its DNA-dependent nucleoside triphosphatase activity provides evidence that the active form of the enzyme, even in this relatively simple hydrolytic reaction, is multimeric (10, 11). It appears certain that the RecA protein-RecA protein interactions that occur between neighboring monomers in these RecA protein-DNA complexes are important for both the structure and function of these key intermediates in recombination and gene regulation.

As part of our analysis of the structure and function of different domains within the multifunctional RecA enzyme, we have developed an affinity support in which RecA protein is covalently coupled to agarose to investigate protein-protein interactions involved in RecA monomer association and to localize the region of the polypeptide responsible for these interactions. In this report, we demonstrate that the specific recognition between free RecA protein subunits and the RecA protein-agarose affinity column likely involves both hydrophobic as well as electrostatic interactions. Using a series of truncated RecA protein fragments, we present evidence that the major determinants for selective binding are localized within the first 90 residues of the RecA protein and demonstrate that this domain of RecA protein is functionally conserved among three heterologous RecA proteins. Our results demonstrate that the RecA protein-RecA protein interactions observed using affinity chromatography are unaffected by treatments that disrupt long protein filaments of RecA protein and probably reflect the binding interactions responsible for more stable, less aggregated forms of the protein (3, 4).

We have also examined the binding properties of RecA protein-DNA complexes, so-called presynaptic complexes, to RecA protein-agarose columns. Presynaptic complexes are formed in the presence of ATP or the nonhydrolyzable analog

There is considerable biochemical and biophysical data demonstrating the unusual structural complexity of *Escherichia coli* RecA protein. Although the *recA* gene encodes a relatively small protein of approximately 37.8 kDa, this protein exists as highly aggregated, multimeric species in solution

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Supported by a Molecular and Cell Biology training grant from the United States Public Health Service.

§ Supported by Research Grant GM 29558 from the National Institutes of Health and a Faculty Research award from the American Cancer Society.

ATP γ S¹ and contain stoichiometric amounts of RecA protein (12). Similar nucleoprotein complexes of RecA protein and DNA direct the proteolytic cleavage of the cellular repressor of SOS genes, LexA protein, and the bacteriophage λ repressor, cI protein, *in vitro* (13, 14).

Our results demonstrate that the binding properties of nucleoprotein complexes of RecA protein differ significantly from those of free RecA protein. When formed with single-stranded circular DNAs, complexes bind to RecA protein-agarose and are eluted with buffers of moderate to high ionic strength, whereas when complexes are formed with oligonucleotides ((dT)₂₅₋₃₀), a considerable fraction of the complexes remain bound in high salt and elute from the affinity column only after treatment with GdnHCl or urea.

EXPERIMENTAL PROCEDURES

Materials—RecA protein was purified to homogeneity from *E. coli* strain KM1842 as described previously (15), and aliquots were stored at -70°C in R buffer (20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol) containing 25% glycerol. RecA protein concentrations were calculated using a value of $E_{280}^{1\%} = 5.16$ (15).

³H-Labeled M13 single-stranded DNA was a generous gift from James Halbrook of this laboratory. Oligodeoxythymidylic acid (oligo(dT)₂₅₋₃₀) was purchased from Pharmacia LKB Biotechnology Inc.; ATP γ S was obtained from Boehringer Mannheim. Nalidixic acid, nucleoside triphosphates, and octyl-agarose were purchased from Sigma; Affi-Gel 10 was purchased from Bio-Rad. The source of ³⁵S (as H₂SO₄) for *in vivo* protein labeling was ICN. The coupled DNA-directed prokaryotic translation kits and L-[³⁵S]methionine were purchased from Amersham Corp. and used as instructed by the manufacturer.

Plasmids containing truncated *recA* genes were constructed in this laboratory by Naoko Kobayashi and are described for Fig. 6. The plasmid pJC917 containing the carboxyl-terminal coding portion of the *recA* gene was provided by Dr. A. J. Clark (Department of Molecular Biology, University of California, Berkeley). This plasmid contained the distal 1.4-kilobase *EcoRI*-*Bam*HI restriction fragment of the *E. coli* K12 *recA* gene inserted into plasmid pUC8-2, thereby fusing in-frame the last 92 codons of the *recA* gene with the first six codons of *lacZ*. The resultant fusion protein is under the control of the *lac* promoter. Expression of this fusion polypeptide in *E. coli* strains containing plasmid pJC917 was demonstrated using a monoclonal antibody (Ab156) specific for an antigenic determinant contained within the last 45 residues of RecA protein (16). Ab156 was a generous gift of Dr. Alex Karu (Naval Biosciences Laboratory, Oakland, CA).

Preparation of RecA Protein Affinity Columns—Purified RecA protein was dialyzed into coupling buffer (0.1 M MOPS (pH 6.8), 5% glycerol) and added to a slurry of Affi-Gel 10 at a concentration of approximately 12 mg of protein/ml of gel. The mixture was rocked gently at 4°C for 12–14 h, and coupling was blocked by the addition of 0.1 ml of 1 M ethanolamine HCl (pH 8)/ml of gel. The mixture was shaken gently for an additional 1–2 h. Following the coupling reaction, the column was washed extensively with 2 M NaCl. Typically, more than 6 mg of RecA protein bound per ml of agarose matrix. A control column containing BSA was prepared essentially as described for RecA protein except that 80 mM CaCl₂ was included in the original coupling step. Affinity columns (1 ml) were prepared in 3-ml plastic syringes and washed extensively with R buffer containing glycerol (5%). Columns were stored at 4°C in R buffer containing glycerol (5%) and sodium azide (0.02%). The binding properties of these columns varied little from preparation to preparation. Columns could be reused after treating with GdnHCl (5 M), washing extensively with R buffer, and allowing to renature at 4°C .

Labeling of Cells and Purification of ³⁵S-Labeled RecA Protein—Strain KM1842 was grown in M9 salts (17) containing 50 μM MgSO₄ and supplemented with 0.4% glucose, 100 μM CaCl₂, 5 $\mu\text{g}/\text{ml}$ thiamine, and 0.2% casamino acids. At a cell density of OD₅₉₅ = 0.1, H₂³⁵SO₄ was added (5 mCi/liter), and the culture was incubated with aeration

until it reached a density of OD₅₉₅ = 0.6–0.7. Nalidixic acid (40 $\mu\text{g}/\text{ml}$) was added to the culture for an additional 60 min, and the labeled cells were collected by brief centrifugation and washed in M9 salts. Crude soluble extracts were prepared using Brij lysis as described (15), treated with RNase and DNase (1 $\mu\text{g}/\text{ml}$) for 1 h at 25°C , and dialyzed into R buffer containing glycerol (5%). Alternatively, the labeled soluble extract was used for purifying RecA protein essentially as described (15).

Plasmid DNA-directed Translation of Truncated RecA Proteins—Plasmid DNAs were purified by centrifugation to equilibrium in CsCl gradients containing ethidium bromide (18). Aliquots (2.5 μg) were added to *in vitro* translation reactions containing L-[³⁵S]methionine according to protocols furnished by the manufacturer (Amersham Corp.). The products of the translation reactions were characterized by electrophoresis in polyacrylamide gels containing SDS and visualized by fluorography.

Affinity Column and Octyl-Agarose Chromatography—Column chromatography was performed at 23°C as follows. Samples of soluble proteins from labeled cells (approximately $1-2 \times 10^8$ cpm) or purified RecA protein (50 μg) were loaded onto columns (1 ml) equilibrated with R buffer containing 5% glycerol and 10 mM MgCl₂ (loading buffer) unless otherwise indicated. After incubation for 30 min, the columns were washed as described in the figure legends, and fractions (0.5 ml) were collected. From each fraction, an aliquot (200 μl) was removed, and radioactivity was determined by liquid scintillation counting. Fractions of interest were also analyzed by electrophoresis in polyacrylamide gels containing SDS (15% acrylamide, 0.4% bis-acrylamide). Following electrophoresis, polyacrylamide gels were stained with Coomassie Brilliant Blue to locate molecular weight standards and soaked in 1 M sodium salicylate (30 min) before drying and fluorography.

Column chromatography of *in vitro* translated proteins was performed by diluting the entire translation reaction mixture (35 μl) with R buffer/glycerol/MgCl₂ (25 μl) and applying the entire volume to the affinity column. After incubation (30 min) at room temperature, the columns were washed with 5 volumes each of loading buffer containing no salt, 200 mM NaCl, 2 M NaCl, and 5 M GdnHCl. Aliquots (250 μl) of each fraction were treated with 1 M NaOH (500 μl) for 30 min at 37°C and precipitated with cold trichloroacetic acid (4 ml, 10%). The precipitated protein was collected onto Whatman GF/C filters, and radioactivity was determined by liquid scintillation counting. Column fractions of interest were analyzed by electrophoresis in polyacrylamide gels and fluorography as described above.

Hydropathy Analysis—The hydropathy profile of RecA protein was performed using the Beckman Microgenie program and an IBM PC/AT computer.

Nucleoprotein Complex Formation and Chromatography—Nucleoprotein complexes of RecA protein, single-stranded DNA, or oligonucleotide and ATP γ S were formed as follows. RecA protein (50 μg) was incubated in a reaction (50 μl) containing 20 mM Tris-HCl (pH 7.5), 20 mM KCl, 10 mM MgCl₂, 5 mM dithiothreitol, 1 mM ATP γ S, and either ³H-labeled M13 DNA or oligo(dT)₂₅₋₇₀ for 30 min at 37°C . DNA was present at a 20-fold molar excess (nucleotides) to RecA protein. Following complex formation, the reaction mixtures were diluted with 250 μl of R buffer containing MgCl₂ (10 mM). These complexes were loaded onto RecA protein-agarose and allowed to stand for 30 min at room temperature. The column was washed with 5 volumes of loading buffer and eluted with increasing concentrations of NaCl in loading buffer.

RESULTS

Specific Binding of RecA Protein to RecA-Agarose Affinity Columns—Protein affinity chromatography is a valuable technique for investigating protein-protein interactions in multienzyme complexes. The technique has been successfully applied to studies of replication and recombination complexes in phage T4-infected cells (19, 20). We reasoned that this technique could be applied to address two questions concerning RecA protein of *E. coli*: (i) what, if any, cellular proteins bind to RecA protein; and (ii) what is the nature of RecA protein-RecA protein interactions that contributes to its oligomerization properties?

Initially, we used the RecA protein-agarose column to investigate binding of cellular proteins from soluble cell extracts prepared from *E. coli*. We applied a crude ³⁵S-labeled cell

¹ The abbreviations used are: ATP γ S, adenosine 5'-O-(thiotriphosphate); BSA, bovine serum albumin; MOPS, 3-(N-morpholino)propanesulfonic acid; SDS, sodium dodecyl sulfate; AmSO₄, ammonium sulfate; GdnHCl, guanidine hydrochloride; ssDNA, single-stranded DNA; Ab, antibody.

extract to an RecA protein-agarose column and eluted the proteins with an NaCl gradient. The profile of eluted radioactivity (Fig. 1A) demonstrated that under these conditions more than 99% of the ^{35}S -labeled proteins did not bind to RecA protein-agarose and were found in the column pass-through. The ^{35}S -labeled proteins that were retained on the affinity column eluted over the entire salt gradient. This radioactive material represents proteins that show a significant interaction with the RecA protein-agarose affinity matrix. We characterized these bound proteins by polyacrylamide gel electrophoresis of individual column fractions. As shown in Fig. 1B, radioactivity in the pass-through fractions contains numerous proteins. In fact, the distribution of radioactive proteins was almost indistinguishable from the load material. Only one protein bound to the RecA protein-agarose column at salt concentrations above approximately 200 mM NaCl, and this protein migrated with the RecA protein marker. The protein eluted over the entire NaCl concentration range of the gradient (0.02–0.5 M) and was further eluted by washing the column with 2 M NaCl. We demonstrated that this bound ^{35}S -labeled protein was RecA protein by showing that it reacted with a monoclonal antibody (Ab156) prepared against purified RecA protein (data not shown).

One other protein was retained on the RecA affinity column and eluted in the NaCl gradient between 50 and 150 mM salt. The size of this protein was estimated to be approximately 25 kDa, and this protein was immunologically distinct from RecA protein. No other proteins bound reproducibly to the column, although occasionally we observed polypeptides with molecular sizes of approximately 30 kDa that were proteolytic fragments of RecA protein as judged by their cross-reaction with anti-RecA antibodies (data not shown).

We prepared an agarose column containing BSA to test for nonspecific retention of RecA protein. The BSA-agarose column was prepared as described under "Experimental Procedures," and a ^{35}S -labeled soluble protein extract was applied to it and eluted with a gradient of NaCl identically to the

RecA protein-agarose column. As shown in Fig. 1 (A and C), all of the labeled proteins were recovered in the pass-through and low salt wash fractions. Although a small amount of radioactivity was recovered in fractions obtained by washing the column at higher NaCl concentrations, no protein bands were visible in column fractions as judged by fluorography (Fig. 1C). These results argue that labeled RecA protein is retained on the RecA protein-agarose columns through RecA protein-RecA protein interactions and not by interactions with the agarose support. Furthermore, the elution properties of labeled RecA protein suggest some heterogeneity in its binding interaction with the affinity column.

We have also examined the binding of several purified proteins from *E. coli* which, based on biochemical and genetic data, might interact with RecA protein *in vivo* but are present in crude extracts in amounts that are too low to be detected by fluorography of labeled proteins. For these investigations, the purified protein was applied to the RecA protein-agarose column using the same conditions as those used for the labeled soluble cell extracts. Proteins in individual column fractions were separated by polyacrylamide gel electrophoresis and visualized by silver staining. No binding of the *E. coli* single-stranded DNA-binding protein, DNA polymerase I, or phage λ repressor (cI protein) has been detected. LexA protein did not bind to the RecA protein-agarose column and was found in the pass-through fractions (data not shown). These results further argue that RecA protein was retained on the affinity column through specific RecA protein-RecA protein interactions.

One possible explanation for the binding of RecA protein in the crude ^{35}S -labeled protein fractions was that this protein was being retained through contaminating DNA or RNA that bound to the immobilized RecA. This possibility was ruled out by extensively treating the cell extracts with RNase and DNase prior to loading onto the RecA protein-agarose column. These treatments did not alter the pattern of protein binding to the affinity column. Moreover, labeled single-

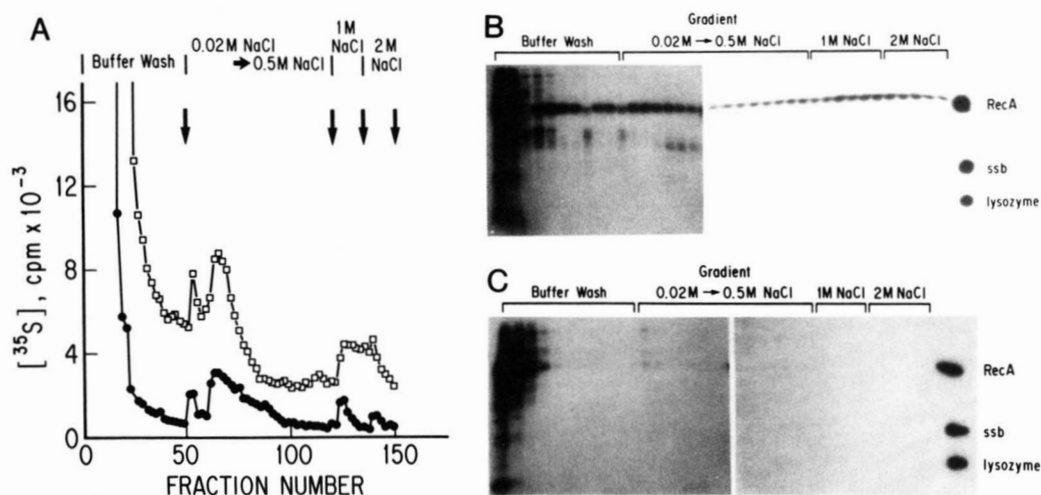


FIG. 1. Binding of soluble proteins from *E. coli* to RecA and BSA protein affinity columns. ^{35}S -Labeled soluble proteins from cells were prepared, and equivalent amounts of radioactivity (1.7×10^6 cpm) were applied to RecA protein-agarose and BSA-agarose columns as described under "Experimental Procedures." Columns were washed with loading buffer (5 ml) and a linear gradient of NaCl (0.02–0.5 M), followed by 1 and 2 M NaCl washes. A, elution profile of ^{35}S from RecA protein-agarose (\square) and BSA-agarose (\bullet) columns. Radioactivity in fractions was determined by liquid scintillation counting. The peak fractions in the buffer wash were 5.2×10^6 cpm for RecA protein-agarose and 8.6×10^6 for BSA-agarose. Recovery from the columns was greater than 95%. B, representative samples from the RecA protein-agarose affinity column profile shown in A were analyzed by polyacrylamide gel (11%) electrophoresis in the presence of SDS and fluorography. RecA protein, single-stranded DNA-binding protein (ssb), and lysozyme markers were visualized by staining with Coomassie Brilliant Blue. C, polyacrylamide gel electrophoresis and fluorography of column fractions from the BSA-agarose column.

stranded DNA did not bind efficiently to RecA protein-agarose columns (see below).

Requirements for RecA Protein Binding to RecA Protein-Agarose—We investigated the requirements for specific retention of RecA protein on RecA protein-agarose columns using ^{35}S -labeled RecA protein which was purified by standard procedures (see "Experimental Procedures") and shown to have DNA-dependent adenosine triphosphatase activity identical to unlabeled RecA protein (data not shown). We loaded this RecA protein onto a RecA protein-agarose column under a variety of conditions and examined the pass-through and bound column fractions. The results are summarized in Fig. 2 and Table I. Quantitative retention of RecA protein to the affinity column required Mg^{2+} ; in its absence, approximately 33% of the protein was recovered in the pass-through fractions (Fig. 2). In all subsequent experiments, MgCl_2 (10 mM) was present in the column buffers to maximize RecA binding. As shown in Table I, the presence of ATP in the column buffer did not affect the amount of RecA protein that bound to the affinity column. Furthermore, the binding occurred equally well at 4 °C and at 23 °C, although the kinetics of binding were not examined at both temperatures. An unexpected finding was that RecA protein bound tightly and quantitatively to the RecA protein-agarose column when it was loaded in the presence of 1.8 M AmSO_4 , which suggests that nonionic forces were in large part responsible for RecA protein binding to the affinity column.

Elution of Bound RecA Protein from RecA Protein-Agarose Affinity Columns—The results shown in Table I indicate that ^{35}S -labeled RecA protein remained bound to the RecA protein-agarose columns in the presence of high salt. Using homogeneous ^{35}S -labeled RecA protein, we calculated that only approximately 50–60% of RecA protein loaded onto the columns was eluted by extensively washing the column with

2 M NaCl. We therefore sought conditions that would remove bound RecA protein. A summary of these experiments is shown in Table II. Although Mg^{2+} was required for quantitative binding to the column, no ^{35}S -labeled RecA protein was eluted when the column was washed extensively (10 column volumes) with 5 mM EDTA. In addition, little or no RecA protein was eluted from RecA protein-agarose columns washed with ATP, single-stranded DNA, or a combination of single-stranded DNA and the nonhydrolyzable ATP analog $\text{ATP}\gamma\text{S}$.

Treatment of bound ^{35}S -labeled RecA protein with loading buffer containing Triton X-100 (1%) released approximately 4% of the radioactivity. We observed that washing the bound material with loading buffer containing 0.5, 4, and 8 M urea released 0, 40, and 92% of ^{35}S -labeled RecA protein, respectively. Similarly, exposing the column to R buffer containing 5 M GdnHCl quantitatively removed labeled RecA protein that was bound to the affinity column.

Hydrophobic Effects Contribute to RecA Protein-RecA Protein Binding—The binding and elution behavior of RecA protein indicated that nonionic interactions were important for protein-protein recognition. Indeed, the ability of RecA protein to bind to RecA protein-agarose columns in the presence of high concentrations of salt suggested that binding might be mediated through hydrophobic effects. ^{35}S -labeled RecA protein was applied to an RecA protein-agarose column equilibrated with 1.8 M AmSO_4 , and the ionic strength was reduced in order to elute bound protein (Fig. 3). As seen earlier, all RecA protein was retained on the column in the high salt-containing buffer; and moreover, little or no RecA protein was eluted throughout the entire range of AmSO_4 concentrations. It was expected that if RecA protein-RecA protein interactions were purely hydrophobic in nature, the decreasing ionic strength would unmask charged groups on the protein and increase charge repulsion between subunits. Because labeled RecA protein remained bound throughout the gradient, it is likely that other forces contribute to subunit binding. Nevertheless, the hydrophobic binding properties of RecA protein could be demonstrated on octyl-agarose columns. RecA protein was quantitatively retained on the octyl-agarose support over a wide range of NaCl concentrations. Removal of the bound protein was achieved using 5 M GdnHCl (or 8 M urea), as was required for elution from the RecA protein-agarose columns (data not shown).

As shown in Fig. 1B, only RecA protein from soluble protein extracts bound tightly to RecA protein-agarose columns, a result that suggests that retention is due to specific interactions. Nevertheless, because RecA protein binds tightly to octyl-agarose, it seemed possible that the RecA affinity col-

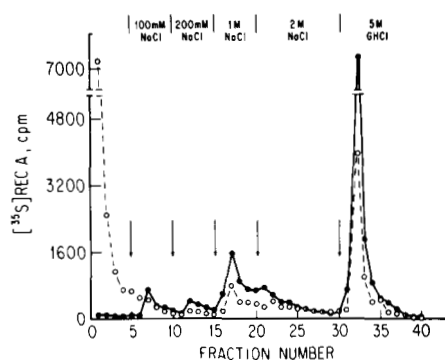


FIG. 2. Efficient binding of RecA protein to RecA protein-agarose columns requires Mg^{2+} . Purified ^{35}S -labeled RecA protein was applied to a RecA protein-agarose column and eluted with the indicated NaCl step gradient in the absence (○) or presence (●) of MgCl_2 (10 mM). Columns were treated with GdnHCl (5 M) to remove all bound protein. Recovery was greater than 95% for each affinity column.

TABLE I
Requirements for RecA protein binding to RecA protein-agarose columns

Treatment	Amount retained ^a
	%
None	(100)
+1 mM ATP	100
+700 μM M13 ssDNA	84

^a The amount of retained was the fraction of ^{35}S -labeled RecA protein which bound to the affinity column and was eluted by 5 M GdnHCl relative to the untreated sample.

TABLE II
Elution of bound ^{35}S -labeled RecA protein from RecA protein-agarose columns

Treatment	Amount remaining ^a
	%
None	(100)
+5 mM EDTA	100
+1 mM ATP	100
+700 μM M13 ssDNA	84
+1 mM $\text{ATP}\gamma\text{S}$, 700 μM M13 ssDNA	75
+1% Triton X-100	96
+0.5 M urea	100
+4 M urea	60
+8 M urea	8
+5 M GdnHCl	0

^a The amount remaining was the amount of ^{35}S -labeled RecA protein retained on the column after the indicated treatment divided by the original amount bound.

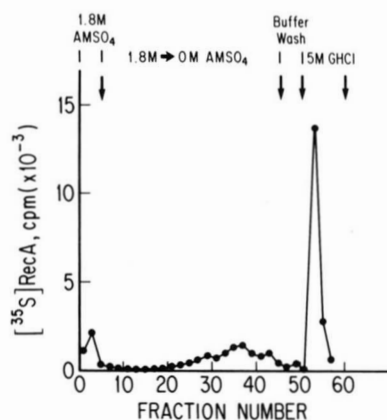


FIG. 3. Binding of RecA protein to RecA protein-agarose column in presence of high salt. Purified ^{35}S -labeled RecA protein was applied to an RecA protein-agarose column equilibrated with loading buffer containing 1.8 M AmSO_4 . The column was washed sequentially with high salt-containing loading buffer (10 column volumes) and a reverse gradient of AmSO_4 . Following this, the column was treated with loading buffer, and remaining RecA protein was eluted with buffer containing 5 M GdnHCl (GHCl). Radioactivity in fractions was determined by liquid scintillation counting.

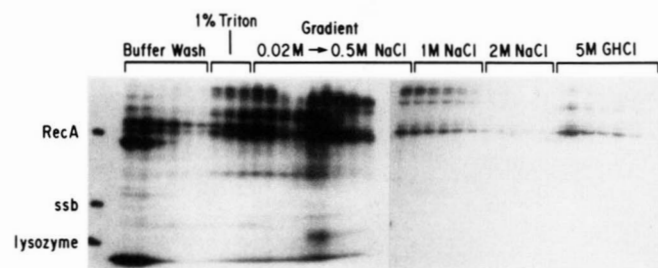


FIG. 4. Binding of *E. coli* soluble proteins to octyl-agarose. ^{35}S -Labeled proteins were prepared from cells and applied to an octyl-agarose column ($1-2 \times 10^8$ cpm). The column was washed with loading buffer (10 ml), buffer containing 1% Triton X-100, and increasing NaCl concentrations as indicated. Treatment with GdnHCl (5 M) was used to remove remaining proteins. Column fractions were analyzed by polyacrylamide gel electrophoresis and fluorography.

umn might retain proteins through nonspecific hydrophobic effects. One way we tested this possibility was to examine the elution pattern of proteins from soluble *E. coli* extracts on octyl-agarose columns. As shown in Fig. 4, the elution profile of ^{35}S -labeled soluble proteins on octyl-agarose columns differed significantly from the profile obtained using RecA protein-agarose columns (see Fig. 1B). In particular, a considerably greater number of labeled proteins bound to the octyl-agarose column and eluted between 20 mM and 0.5 M NaCl. As expected, RecA protein in the crude protein extract was retained on the octyl-agarose column and eluted over a wide range of salt concentrations. Treatment of the column with 5 M GdnHCl was required for complete removal of RecA protein. These results argue that the selective retention of RecA protein on RecA protein-agarose was not due to the RecA protein-agarose column acting as a nonspecific hydrophobic affinity column such as octyl-agarose since several *E. coli* proteins showed a strong affinity for octyl-agarose but did not bind to the RecA protein-agarose column. Moreover, binding of proteins from crude extracts to octyl-agarose did not require Mg^{2+} (data not shown), a result that further supports the idea that specific protein-protein interactions are responsible for retention of RecA protein on RecA protein-agarose columns.

Amino-terminal Domain of RecA Protein Participates in Protein-Protein Recognition—Our results indicated that bind-

ing of RecA protein subunits is mediated in part through hydrophobic interactions. We wished to determine what region or regions of RecA protein are required for the strong binding we observed. As a first step, we searched the primary sequence of RecA protein for regions of unusual hydrophobicity using the method of Hopp and Woods (21). The hydrophathy profile for wild-type RecA protein is shown in Fig. 5. Inspection of the profile suggested that there are three regions of extended hydrophobic character in the primary structure of RecA protein: residues 35–85, 185–220, and 255–295. In order to determine whether these or other regions of RecA protein are required for protein-protein recognition, we constructed truncated *recA* genes in the plasmid vector pBR322 and used these DNAs to direct synthesis *in vitro* of truncated RecA polypeptides. The plasmids used in the coupled transcription-translation reaction are depicted in Fig. 6. Plasmids pNK100 and pNK200 directed synthesis of polypeptides containing the first 90 and 258 residues of RecA protein, respectively. Because there are no translational termination sequences at the ends of the cloned fragments, these peptides terminate within plasmid sequences. Based on analysis of the downstream nucleotide sequence, we predicted that the polypeptide directed from plasmid pNK100 would contain 16 additional amino acids at its carboxyl terminus, whereas plasmid pNK200 would direct synthesis of a polypeptide containing an additional 7 residues at its carboxyl terminus. Plasmid pJC917 contains an in-frame fusion of the first six codons of LacZ protein with the last 92 codons of RecA protein. This plasmid directs synthesis of a LacZ-RecA fusion polypeptide controlled from the *lac* promoter. In addition, we used plasmid pBR recA (22) to direct synthesis of full-length RecA protein, which served as a positive control for affinity column binding. The binding of RecA protein synthesized *in vitro* to the RecA protein-agarose affinity column is shown in Fig. 7. Although several other proteins encoded by the plasmid were synthesized *in vitro*, only RecA protein bound to the RecA protein-agarose column. The product of the ampicillin resistance gene β -lactamase, with a molecular size of 27 kDa, migrated ahead of RecA protein in polyacrylamide gels containing SDS. Unlike purified RecA protein, which bound quantitatively to RecA protein-agarose, RecA protein synthesized *in vitro* did not bind quantitatively to the affinity

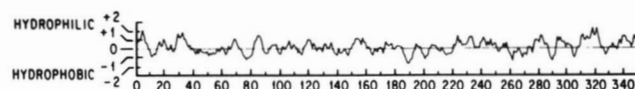


FIG. 5. Hydrophathy profile of RecA protein. The method of Hopp and Woods (21) was used to predict hydrophilic (positive values) and hydrophobic (negative values) regions along the length of the RecA polypeptide chain.

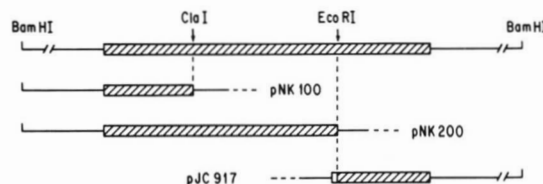


FIG. 6. Plasmids containing truncated and fused *recA* genes. Plasmids used for directing *in vitro* protein synthesis were prepared by digesting the 3.0-kilobase *Bam*HI fragment containing the *recA* gene of *E. coli* with *Cla*I or *Eco*RI, each of which cuts once in the *recA* coding sequence (hatched boxes). Plasmids pNK100 and pNK200 contain the *Bam*HI-*Cla*I and *Eco*RI-*Bam*HI fragments, respectively, cloned into pBR322. Plasmid pJC917 contains the *lac* operator and promoter and six amino terminal codons (open box) fused in-frame to the *recA* coding sequence at the *Eco*RI site.

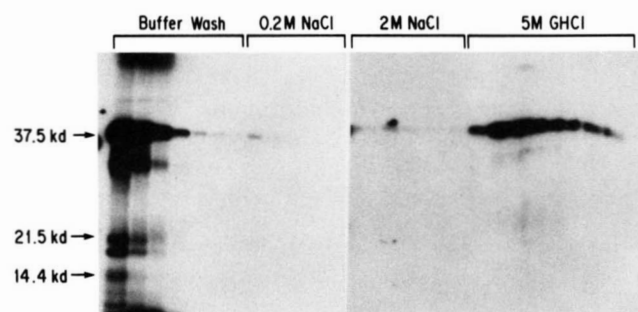


FIG. 7. Binding of RecA protein synthesized *in vitro* to RecA protein-agarose column. ^{35}S -Labeled RecA protein was synthesized *in vitro* using pBRrecA as template. The unfractionated translation reaction was applied to a RecA protein-agarose column and washed with increasing NaCl concentrations as shown. The column was treated with GdnHCl (5 M) to remove remaining RecA protein. Column fractions were analyzed by polyacrylamide gel electrophoresis and fluorography. The smaller labeled proteins that are present in the buffer wash fractions are likely encoded by plasmid sequences.

TABLE III

Binding of *in vitro* synthesized RecA protein and truncated RecA polypeptides to RecA protein-agarose columns

Plasmid	Residues	Fraction bound ^a
pBRrecA	1-352	(100)
pNK100	1-90	100
pNK200	1-258	47
pJC917	258-352	0

^a The fraction bound was the fraction of ^{35}S -labeled RecA fragment bound compared to the fraction of full-length RecA protein that bound to RecA protein-agarose columns.

column, and a considerable fraction was found in the pass-through. Because this RecA protein was loaded onto the affinity column together with the unlabeled components of the coupled protein synthesis system (*i.e.* S30 extract), we tested whether these cell extracts contained an inhibitor of RecA protein binding. Purified ^{35}S -labeled RecA protein was mixed with an amount of unlabeled crude cell extract comparable to that used in the synthetic reactions, and the mixture was applied to an RecA protein-agarose column. Analysis of the resultant column fractions demonstrated that approximately 60% of labeled RecA protein was eluting in the pass-through fractions, and the remainder bound tightly to the column. In the absence of cell extract, almost 100% of the radioactivity remained bound to the column, consistent with earlier results (see Fig. 2). We conclude that soluble cell extracts contain one or more inhibitors that reduce binding of RecA protein to the RecA protein-agarose column. Despite the presence of these inhibiting factors, it was possible to detect binding of the *in vitro* translation products to the RecA protein-agarose column.

The binding properties of the truncated RecA polypeptides are summarized in Table III. In addition to full-length RecA protein, two polypeptides containing different amounts of the amino-terminal region of RecA protein bound tightly to the RecA protein-agarose column. The smaller polypeptide, containing 90 residues of RecA protein, bound to the column as well as the full-length polypeptide synthesized *in vitro*. A protein fragment containing approximately two-thirds of the protein residues bound less well than the smaller truncated product. By way of contrast, the carboxyl-terminal polypeptide encoded by plasmid pJC917 did not bind to the affinity column and was completely recovered in the pass-through fractions. We conclude that a region(s) within the first 90 residues of RecA protein is sufficient for binding to the

affinity column and is likely to be necessary for subunit interactions. These results are consistent with the genetic properties of truncated *recA* gene fragments *in vivo* (see "Discussion").

Heterologous RecA Proteins Bind Tightly to RecA Protein-Agarose Columns—We have cloned a series of *recA* genes derived from several Gram-negative bacteria. These proteins are expressed in *E. coli* K12 and functionally complement several of the defects associated with an *recA* deficiency. We have shown that these proteins display considerable conservation of protein structure based upon immunological cross-reactivity (22) and similarity of tryptic peptides (23). In order to determine whether these proteins could interact with RecA protein from *E. coli* K12, we prepared ^{35}S -labeled soluble extracts from strain JC14604 containing a deletion of the *recA* gene and carrying plasmids encoding the heterologous *recA* counterpart. Cell extracts were prepared containing RecA proteins from *Proteus vulgaris*, *Shigella flexneri*, and *Erwinia carotovora* and analyzed for binding to the RecA protein-agarose column. In all cases, we observed specific retention of heterologous RecA protein on the affinity column. The *S. flexneri* column profile is shown in Fig. 8. As is seen with the *E. coli* K12 RecA protein, complete removal of the heterologous protein required treatment with chaotropic agents such as GdnHCl. These results indicate that the site(s) of RecA protein which participate in protein recognition and binding have been functionally conserved.

Binding of Nucleoprotein Complexes to RecA Protein-Agarose Columns—Because the binding of RecA protein to DNA can result in hydrolysis of nucleoside triphosphates, pairing of homologous DNA sequences, or proteolytic cleavage of specific viral and cellular repressors, we decided to investigate the interaction of RecA protein-DNA complexes with the RecA protein-agarose columns. Fig. 9 (*upper left panel*) shows an elution profile of ^3H -labeled M13 single-stranded DNA on the RecA protein-agarose column. Greater than 99% of the radioactivity was recovered in the pass-through fractions. This result demonstrates that immobilized RecA protein cannot bind free DNA directly. In a second experiment (*upper right panel*), ^3H -labeled M13 DNA was incubated with ^{35}S -labeled RecA protein prior to loading onto the affinity column. A 20-fold molar excess of DNA was used to accommodate binding of all labeled RecA protein. In contrast to the results obtained when RecA protein alone was loaded onto the RecA protein-agarose columns, approximately 33% of ^{35}S -labeled RecA protein was found in the pass-through and 0.1 M NaCl elution fractions. More than 93% of the ^3H -labeled M13 DNA

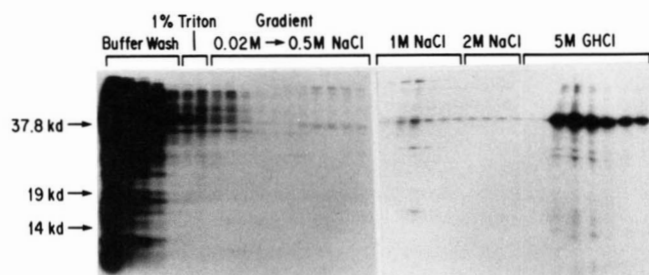


FIG. 8. Specific binding of *S. flexneri* RecA protein to RecA protein-agarose columns. *E. coli* strain JC14604 containing plasmid pMK816 (22) was labeled with ^{35}S , and a soluble protein extract was prepared as described under "Experimental Procedures." The protein mixture was applied to a RecA protein-agarose column and sequentially washed with loading buffer, buffer containing 1% Triton X-100, a linear 0.02–0.5 M gradient of NaCl, 1 M NaCl, 2 M NaCl, and 5 M GdnHCl. Column fractions were analyzed by polyacrylamide gel electrophoresis and fluorography.

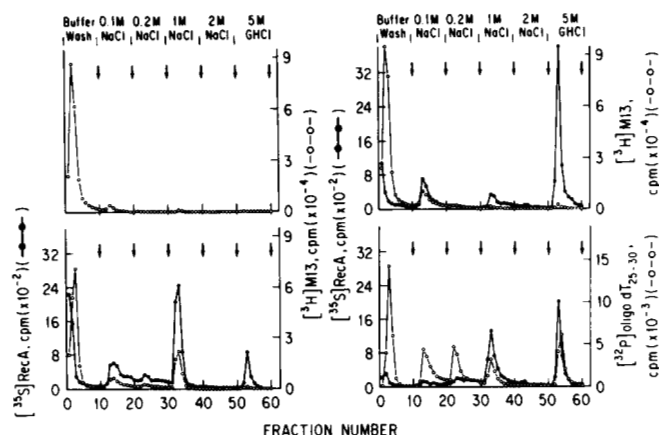


FIG. 9. RecA protein affinity chromatography of RecA protein-DNA complexes. Upper left panel, ^3H -labeled M13 ssDNA (50 nmol) was applied to a RecA protein-agarose column (1 ml) and incubated for 15 min, and the column was washed with buffer containing increasing NaCl concentrations (0.1–2 M) as shown (5 column volumes/step). After the salt washes, the column was treated with GdnHCl (5 M). Radioactivity in individual column fractions was determined by liquid scintillation counting. Upper right panel, ^{35}S -labeled RecA protein (2.5 nmol) and ^3H -labeled M13 DNA (50 nmol) were incubated together (15 min, 37°C) and applied to a RecA protein-agarose column (1 ml). The column was washed as indicated, and radioactivity in individual fractions was determined by scintillation counting. Lower left panel, ^{35}S -labeled RecA protein (2.5 nmol), ^3H -labeled M13 ssDNA (50 nmol), and ATP γ S (1 mM) were formed as described under "Experimental Procedures," loaded onto a RecA protein-agarose column (1 ml), and washed as indicated. Radioactivity was determined by liquid scintillation counting. Lower right panel, ^{35}S -labeled RecA protein (2.5 nmol) was preincubated with oligo(dT) $_{25-30}$ (50 nmol) and ATP γ S (1 mM) and applied to a RecA protein-agarose column (1 ml) as described above. In a separate experiment performed under identical conditions, unlabeled RecA protein was preincubated with ^{32}P -labeled oligo(dT) $_{25-30}$ and applied to a RecA protein-agarose column. The combined radioactivity profiles are shown.

was recovered in the pass-through and 100 mM NaCl wash fractions. Because RecA protein freely dissociates from single-stranded DNA, we interpreted this result to mean that RecA protein-DNA complexes bound weakly to RecA protein-agarose columns, whereas RecA protein that was not complexed with DNA bound tightly. This unbound RecA protein was retained on the RecA protein-agarose column and was eluted with 5 M GdnHCl as we have observed previously. In order to test this explanation, we prepared nucleoprotein complexes of ^{35}S -labeled RecA protein, ^3H -labeled M13 DNA, and ATP γ S as described under "Experimental Procedures." In the presence of ATP γ S, RecA protein binds extremely tightly to DNA and dissociates slowly even in the presence of high salt. ATP γ S is also retained in the complex, with one molecule of analog bound per molecule of RecA protein (24). The elution properties of the RecA protein-DNA complexes formed in the presence of ATP γ S are shown in Fig. 9 (lower left panel). These complexes eluted from the RecA protein-agarose column in the pass-through fractions as well as over a wide range of NaCl concentrations (0.1–1 M). More than 90% of RecA protein was recovered in these fractions, whereas only 9% remained bound and was removed by treating the column with GdnHCl (5 M). The multiple peaks of RecA protein in the elution profile suggested to us that there was considerable heterogeneity in the nucleoprotein complexes and likely reflect different RecA protein-DNA binding stoichiometries. We calculated the molar ratio of RecA protein and DNA using the specific activities of the preparations and found that in complexes eluted with 1 M NaCl, the RecA

protein:DNA molar ratio was 1:4, a value which is close to binding saturation (2, 25). Thus, it appears that when RecA protein was tightly bound to M13 DNA in a protein-DNA complex, it bound to RecA protein-agarose columns primarily through electrostatic, rather than hydrophobic, interactions.

We also examined the binding of complexes containing RecA protein, ATP γ S, and an oligonucleotide consisting of 25–30 deoxythymidylate residues ((dT) $_{25-30}$). This oligonucleotide bound RecA protein and served as an efficient co-factor for ATP hydrolysis by the enzyme (data not shown). When preformed complexes of RecA protein, oligo(dT) $_{25-30}$, and ATP γ S were bound to RecA protein-agarose columns, we obtained the elution profile shown in Fig. 9 (lower right panel). Unlike complexes containing M13 DNA, the oligonucleotide-containing complexes were almost completely retained on the column, with only about 8% of RecA protein recovered in the pass-through. More than 60% of the labeled protein was eluted between 100 mM and 1 M NaCl, with more than 35% of the RecA protein-(dT) $_{25-30}$ complexes remaining bound until the 1 M NaCl wash. The remaining 32% of RecA protein was released following treatment with GdnHCl. By using ^{32}P -labeled oligo(dT) $_{25-30}$, we found that a considerable fraction of the oligonucleotide (17%) was retained on the RecA protein-agarose column when complexed with RecA protein and was eluted by washing with 5 M GdnHCl. These results suggest that some of the RecA protein-oligonucleotide complexes remained on the RecA protein-agarose column and were removed only by washing with GdnHCl. The differences between the protein-protein binding properties of nucleoprotein complexes formed with M13 ssDNA and those formed with the oligonucleotide (dT) $_{25-30}$ may be due to differences in the accessibility of a hydrophobic face of RecA protein when it is bound to these different DNA cofactors (see "Discussion").

DISCUSSION

Structural and biochemical characterization of RecA protein of *E. coli* suggests that this multifunctional protein can exist in different oligomeric forms or aggregation states. We have used RecA protein affinity chromatography to investigate the self-association properties of the enzyme as well as its possible interaction with other cellular proteins. The technique of protein affinity chromatography has recently been employed to investigate the binding of phage T $_4$ -encoded uvsX protein with other phage and host proteins. uvsX protein, like RecA protein, catalyzes synthesis and pairing of homologous DNAs *in vitro* and performs a central role in homologous recombination *in vivo*. The results of Formosa *et al.* (19, 20) indicate that uvsX protein associates with four to five phage proteins as judged by their specific retention on uvsX protein-agarose columns. Genetic and biochemical data argue that these interactions with the affinity column reflect functionally significant physical associations *in vivo*. For example, the dda protein, an ATP-dependent DNA helicase needed for T $_4$ DNA replication, binds to uvsX protein affinity columns and specifically stimulates strand exchange promoted by uvsX protein *in vitro* (26). It is likely that uvsX protein and the dda helicase are part of the protein complex needed for recombination-dependent initiation of late T $_4$ DNA synthesis *in vivo* (27).

Using RecA protein affinity columns, we found evidence for tight binding of a single protein from *E. coli* extracts which we have identified as RecA protein. A second protein, having a molecular size of approximately 25 kDa, bound weakly to the affinity column and eluted between 50 and 150 mM NaCl. (In other experiments, we have observed binding of other

proteins, but these associations have not been reproducible.) The interaction of this protein with RecA protein may have functional significance. However, additional experiments will be necessary to determine whether this protein interacts with RecA protein *in vivo*.

We have also examined the binding of several *E. coli* proteins that, based upon genetic and biochemical data, might interact with RecA protein. We have found no evidence for binding of single stranded DNA-binding protein to RecA protein affinity columns. Although this protein stimulates DNA pairing reactions catalyzed by RecA protein, it does not act by forming a complex with the free enzyme (28, 29).² This result contrasts with the binding of gene 32 protein, the single-stranded DNA-binding protein of phage T₄, to uvsX protein affinity columns (19, 20). Additionally, we found no evidence for binding of DNA polymerase I to RecA protein affinity columns. The cellular repressor LexA did not bind to RecA protein-agarose columns and was eluted in the buffer wash.

The results shown in Fig. 1B demonstrate a strong physical interaction between RecA protein from crude extracts and RecA protein-agarose columns. The elution profile obtained with labeled RecA protein suggests that multiple binding modes are responsible for retention of RecA protein since this protein elutes over the entire NaCl concentration range from 20 mM to 2 M. Nevertheless, this interaction appears specific based on the following arguments. (i) With one exception, all other cellular proteins do not bind RecA protein-agarose columns; and (ii) RecA protein does not bind to a nonspecific affinity column containing bovine serum albumin. We used RecA protein-agarose columns to investigate the requirements for RecA protein subunit binding and the nature of this interaction. In solution, RecA protein is highly aggregated and, under suitable salt and pH conditions, can oligomerize into helical filaments extending up to 1 μ m (3, 4, 6). There is considerable biochemical and structural data demonstrating that the oligomeric structures of RecA protein are important determinants of its enzymatic properties and may represent different functional forms of the enzyme (30–32).

Using highly purified ³⁵S-labeled RecA protein, we have examined the binding requirements and conditions for dissociating RecA protein from the column. Efficient binding requires a divalent cation and occurs over a broad range of temperatures and salt concentrations. In the absence of Mg²⁺, more than 33% of RecA protein fails to bind the RecA column. The binding of RecA protein to RecA protein-agarose in the presence of 1.8 M AmSO₄ argues that association of the protein is mediated at least in part by nonionic interactions. This conclusion is consistent with the elution behavior of bound RecA protein: only chaotropic agents such as urea (8 M) and GdnHCl (5 M) completely remove bound RecA protein. Furthermore, purified RecA protein binds to a hydrophobic affinity column, octyl-agarose, and can be eluted by the same agents. Thus, RecA protein has one or more hydrophobic regions that participate in protein binding.

Based on these results, we asked the question, does RecA protein-agarose retain RecA protein through nonspecific hydrophobic interactions? In order to answer this question, we examined binding of labeled soluble *E. coli* proteins to octyl-agarose columns. The results shown in Fig. 4 demonstrate that a large number of proteins bind this affinity column in contrast to the binding profile on RecA protein-agarose columns (Fig. 1B). We propose that the retention of RecA protein on RecA protein-agarose columns is due to specific protein subunit recognition and that both hydrophobic and electrostatic interactions stabilize the binding. In solutions of

low ionic strength, RecA protein subunits would be held together through electrostatic interactions. However, as the ionic strength of the solution increased, charged groups responsible for these interactions would become shielded, and subunit interactions would be maintained by hydrophobic regions of the protein. Because of this dual binding mode, RecA protein subunit associations can be disrupted at neutral pH only by chaotropic agents such as GdnHCl or urea.

Using truncated *recA* genes, we have prepared labeled polypeptides *in vitro* containing different portions of RecA protein. We examined the ability of these fragments to bind to RecA protein-agarose columns. Our results argue that residues within the first 90 amino acids of the amino terminus are sufficient for RecA protein recognition and binding. Within the shorter amino-terminal fragment that is retained on the affinity columns, there are two regions displaying distinct hydrophobic character based on the analysis shown in Fig. 5. The longer of these regions extends for 23 residues (residues 37–59) and has the following sequence: Val-Glu-Thr-Ile-Ser-Thr-Gly-Ser-Leu-Ser-Leu-Asp-Ile-Ala-Leu-Gly-Ala-Gly-Gly-Leu-Pro-Met-Gly. The second region of hydrophobic amino acids is shorter and extends from residues 73 to 83. The sequence of this region is: Thr-Thr-Leu-Thr-Leu-Gln-Val-Ile-Ala-Ala-Ala. At this time, we do not know whether either or both of these sequences participate in RecA protein-RecA protein binding. These biochemical results using RecA protein affinity chromatography are in agreement with the genetic results of Yarranton and Sedgwick (33), who demonstrated that *recA* gene fragments containing approximately 20% of the promoter proximal coding sequence could negatively complement the *recA*⁺ gene with respect to DNA repair and recombination *in vivo*. This negative complementation likely results from formation of mixed oligomers containing both normal and truncated subunits and which are deficient in several RecA functions. Additionally, there is evidence that proteolysis of the amino-terminal region of RecA protein prevents its aggregation in solution (34).

We examined the binding of three heterologous RecA proteins to *E. coli* RecA protein-agarose columns. Each RecA protein from *P. vulgaris*, *E. carotovora*, and *S. flexneri* binds tightly to the affinity column and shows similar elution properties, suggesting that RecA protein subunit binding interactions have been functionally conserved. Recently, Sedgwick and Goodwin (35) demonstrated that a truncated *recA* gene from *E. coli* K12 encoding the first 211 amino acid residues confers radiosensitivity to several different Gram-negative bacterial species. These genetic results are consistent with the strong physical interactions observed between *E. coli* K12 RecA protein and RecA homologs from three different bacterial strains.

We chose to examine the binding properties of RecA protein-DNA complexes on RecA affinity columns. In particular, we investigated the binding properties of complexes containing RecA protein, single-stranded DNA, and ATP γ S (stable ternary complexes). Nucleoprotein complexes containing stoichiometric amounts of RecA protein stimulate unwinding of duplex DNA and promote limited amounts of pairing between homologous DNAs (31). Furthermore, these complexes participate in the proteolytic cleavage of a specific set of proteins including lambdaoid repressors and LexA and umuD proteins. The biochemical properties of these filaments *in vitro* suggest that they represent the active form of RecA protein *in vivo* during recombination and recovery from DNA damage (31).

Our results demonstrate that the RecA protein-RecA protein binding interactions were altered significantly when the enzyme was associated with DNA. As demonstrated in Fig. 9,

² K. McEntee, unpublished results.

a substantial fraction of RecA protein-DNA complexes eluted from RecA protein-agarose columns after washing the columns with buffer containing NaCl. These results suggest that the hydrophobic regions of RecA protein are partially masked or unavailable in the nucleoprotein filaments. However, it is important to note that there were differences in the elution properties of RecA protein-DNA complexes formed with M13 viral DNA compared to those formed with the oligonucleotide (dT)₂₅₋₃₀. The RecA protein-M13 DNA complexes were eluted by high salt, whereas a significant fraction of complexes formed with the oligonucleotide were retained after high salt washes and were eluted by GdnHCl treatment. Moreover, these latter nucleoprotein complexes were retained on octyl-agarose, demonstrating that hydrophobic surfaces of the protein are available to interact with this column (data not shown).

A simple model consistent with these affinity binding results is shown in Fig. 10. RecA protein binding to single-stranded DNA (polynucleotide or oligonucleotide) is electrostatic and is mediated through binding region 1. Menetski and Kowalczykowski (36) have investigated this interaction in considerable detail and have demonstrated that nucleotide binding to RecA protein modulates the affinity of this interaction: ATP (or ATP γ S) enhances the affinity of RecA protein for DNA, whereas ADP reduces the affinity. We have examined only tightly bound RecA protein complexes formed with the nonhydrolyzable analog ATP γ S. Although these results pertain to these high affinity complexes, it is likely that the basic features of this model are applicable to subunit interactions in the absence of nucleotides. Binding regions 2 and 4 of RecA protein provide the hydrophobic surfaces that govern the interaction of contiguous RecA monomers. It is likely that this interaction plays an important role in the binding of free RecA protein to the affinity column and in the aggregation properties of RecA protein in solution. A "lateral" electrostatic interaction between RecA protein monomers bound to different DNA strands is depicted to occur through region 3 of the protein and is facilitated by salt bridges. This interaction is likely to be crucial for synapse of DNA molecules during recombination and is responsible for the aggregation of RecA protein-DNA filaments that has been observed (37). As with the hydrophobic interaction, we propose that this electrostatic subunit interaction can occur between free RecA subunits. Thus, in the absence of DNA, RecA protein subunits interact through regions 2 and 4 as well as through region 3. In low salt solutions, when ionic

effects are favored, subunit binding is dominated by region 3, whereas in high salt solutions, RecA subunit binding is mediated primarily through hydrophobic effects (regions 2 and 4).

A feature of this model is that only the RecA protein subunits located at the termini of clusters of protein molecules present an accessible hydrophobic surface for binding to RecA protein-agarose columns. Thus, for complexes of RecA protein cooperatively bound to circular DNAs, only a small molar fraction of RecA protein can interact in this manner, whereas when RecA protein is complexed with short oligonucleotides, a substantial fraction of subunits are located at the ends of clusters and can bind RecA protein-agarose through hydrophobic forces. RecA protein-M13 DNA complexes are retained on RecA protein-agarose primarily through electrostatic interactions in the presence of Mg²⁺ (region 3), whereas RecA protein-oligo(dT) complexes also bind through hydrophobic interactions at the ends of the short filaments.

This binding model is consistent with other observations of RecA protein-DNA binding. The cooperativity parameter (ω), as a measure of the cluster size of RecA subunits bound to DNA, has been shown to be independent of ionic strength (36). This observation is consistent with the hydrophobic nature of the binding interaction between contiguous RecA subunits. This model also explains the ability of RecA protein to stimulate ligation of DNAs (38): RecA subunits bound at the termini of DNA chains are brought together by hydrophobic forces and bring DNA ends into proximity for ligation.

The hydrophobic character of RecA protein is surprising in view of its cytoplasmic location in cells, solution properties, and purification behavior. Using hydrophobic interaction chromatography, Alpert (39) has recently demonstrated that RecA protein interacts strongly and somewhat heterogeneously with poly(propyl aspartamide)-silica, indicating that the protein has one or more hydrophobic surfaces. Formosa *et al.* (19, 20) have observed that uvsX protein binds tightly to a uvsX protein-agarose column and can be completely eluted from the affinity matrix by washing with SDS. uvsX protein displays many of the aggregation and association properties described for RecA protein (40). Thus, the strong hydrophobic protein-protein interactions observed with these enzymes may be a general property of enzymes that promote pairing of DNA molecules. Finally, Garvey *et al.* (41) have presented evidence that a significant fraction of RecA protein in damaged cells is associated with cellular membranes and cannot be dislodged by washing in high salt solutions. This observation may reflect the direct interaction of the hydrophobic domain(s) of RecA protein with the cellular membrane or other membrane components.

Acknowledgments—We thank Naoko Kobayashi and Dr. A. J. Clark for plasmids. We thank Janis Young of this Department for assistance with the hydropathy analysis of RecA protein. We are indebted to Janet Ransom and Larry Tabata for their help in preparing this manuscript.

REFERENCES

- Ogawa, T., Wabiko, H., Tsurimoto, T., Horii, T., Masukata, H., and Ogawa, H. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43**, 909-915
- McEntee, K., Weinstock, G. M., and Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 8835-8844
- Flory, J., and Radding, C. M. (1982) *Cell* **28**, 747-756
- Williams, R. C., and Spengler, S. J. (1986) *J. Mol. Biol.* **187**, 109-118
- Morrill, S. W., and Cox, M. M. (1985) *Biochemistry* **24**, 760-767
- Cotterill, S. M., and Fersht, A. R. (1983) *Biochemistry* **22**, 3525-3531

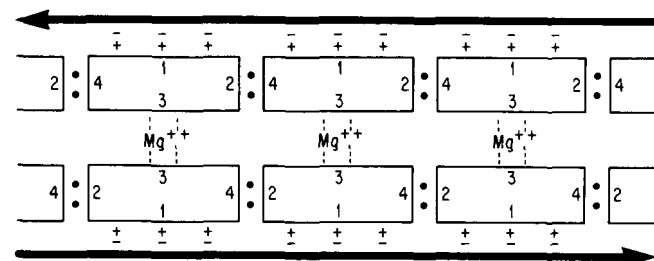


FIG. 10. Binding interactions in RecA protein-DNA complexes. RecA protein monomers bind to single-stranded DNA (thick lines) electrostatically through region 1 of the polypeptide. The interaction of contiguous protein subunits is mediated through hydrophobic regions 2 and 4. Region 3 of RecA protein participates in a lateral subunit interaction which is facilitated by Mg²⁺ salt bridges as shown. Mg²⁺ is required for quantitative binding of RecA protein to the affinity column (see "Results") and promotes the formation of paracrystalline fibers of RecA protein in solution (37). DNA chains are shown in an antiparallel arrangement during synapses. An ATP molecule (or ATP γ S) is bound to each monomer (not shown).

7. Dunn, K., Chrysogelos, S., and Griffith, J. (1982) *Cell* **28**, 757-765
8. Craig, N. L., and Roberts, J. W. (1981) *J. Biol. Chem.* **256**, 8039-8044
9. Pugh, B. F., and Cox, M. M. (1987) *J. Biol. Chem.* **262**, 1326-1336
10. Weinstock, G. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 8845-8849
11. Knight, K. L., and McEntee, K. (1985) *J. Biol. Chem.* **260**, 10177-10184
12. Cunningham, R. P., Shibata, T., Das Gupta, C., and Radding, C. M. (1979) *Nature* **281**, 191-195
13. Little, J. W., Edmiston, S. H., Pacelli, L. Z., and Mount, D. W. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 3225-3229
14. Craig, N. L., and Roberts, J. W. (1980) *Nature* **283**, 26-29
15. Cox, M. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 4676-4678
16. Kobayashi, N., Knight, K. L., and McEntee, K. (1987) *Biochemistry* **26**, 6801-6810
17. Davis, R. W., Botstein, D., and Roth, J. R. (1980) *Advanced Bacterial Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Formosa, T., Burke, R. L., and Alberts, B. M. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80**, 2442-2446
20. Formosa, T., and Alberts, B. M. (1984) *Cold Spring Harbor Symp. Quant. Biol.* **49**, 363-370
21. Hopp, T. P., and Woods, K. R. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78**, 3824-3828
22. Keener, S. L., McNamee, K. P., and McEntee, K. (1984) *J. Bacteriol.* **160**, 153-160
23. Knight, K. L., Hess, R. M., and McEntee, K. (1988) *J. Bacteriol.* **170**, 2427-2432
24. Weinstock, G. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* **256**, 8850-8855
25. West, S. C., Cassuto, E., Mursalim, J., and Howard-Flanders, P. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77**, 2569-2573
26. Kodadek, T., and Alberts, B. M. (1987) *Nature* **326**, 312-314
27. Formosa, T., and Alberts, B. M. (1986) *Cell* **47**, 793-806
28. Soltis, D. A., and Lehman, I. R. (1983) *J. Biol. Chem.* **258**, 6073-6077
29. Muniyappa, K., Shaner, S. L., Tsang, S. S., and Radding, C. M. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 2757-2761
30. McEntee, K. (1985) *Biochemistry* **24**, 4345-4351
31. Cox, M. M., and Lehman, I. R. (1987) *Annu. Rev. Biochem.* **56**, 229-262
32. Sedgwick, S. G., and Yarranton, G. T. (1982) *Mol. Gen. Genet.* **185**, 93-98
33. Yarranton, G. T., and Sedgwick, S. G. (1982) *Mol. Gen. Genet.* **185**, 99-104
34. Kawashima, H., Horii, T., Ogawa, T., and Ogawa, H. (1984) *Mol. Gen. Genet.* **193**, 288-292
35. Sedgwick, S. G., and Goodwin, P. A. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 4172-4176
36. Menetski, J. P., and Kowalczykowski, S. (1985) *J. Mol. Biol.* **181**, 281-295
37. Williams, R. C., and Spengler, S. J. (1986) *J. Mol. Biol.* **187**, 109-118
38. Register, J. C., and Griffith, J. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 624-628
39. Alpert, A. J. (1986) *J. Chromatogr.* **359**, 85-97
40. Formosa, T., and Alberts, B. M. (1986) *J. Biol. Chem.* **261**, 6107-6118
41. Garvey, N., St. John, A. C., and Witkin, E. M. (1985) *J. Bacteriol.* **163**, 870-876