Probing the Functional Role and Localization of Escherichia coli Ribosomal Protein L16 with a Monoclonal Antibody*

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A monoclonal antibody specific for Escherichia coli ribosomal protein L16 was prepared to test its effects on ribosome function and to locate L16 by immunoelectro microscope. The antibody recognized L16 in 50 S subunits, but not in 70 S ribosomes. It inhibited association of ribosomal subunits at 10 mM Mg2+, but not at 15 mM Mg2+. Poly(U)-directed polyphenylalanine synthesis and peptidyltransferase activities were completely inhibited when the L16 antibody was bound to 50 S subunits at a molar ratio of 1. There was no inhibitory effect on the binding of elongation factors or on the associated GTPase activities. Fab fragments of the antibody gave the same result as the intact antibody. Chemical modification of the single histidine (His13) by diethyl pyrocarbonate destroyed antibody binding. Electron microscopy of negatively stained antibody subunit complexes showed antibody binding beside the central protuberance of the 50 S particle on the side away from the L7/L12 stalk and on or near the interface between the two subunits. This site of antibody binding is fully consistent with its biochemical effects that indicate that protein L16 is essential for the peptidyltransferase activity of protein biosynthesis and is at or near the subunit interface.

The peptidyltransferase domain of Escherichia coli ribosomes is contained within the 50 S ribosomal subunit, where both RNA and proteins contribute to its essential properties. L16 is one of the indispensable components of peptidyltransferase. It is one of a small group of ribosomal proteins shown by single protein omission in reconstitution to be required for peptidyltransferase activity (1, 2). Chemical modification of L16 at its single histidine residue at position 13 greatly reduces peptide bond formation (3). We are not aware of any reports of mutant strains that grow without producing L16 (e.g. see Ref. 4). We have used protein-protein cross-linking to map the spatial arrangement of proteins in ribosomal subunits and 70 S ribosomes (5–8); the cross-linking map of L16 shows it to be proximal to L2 and L23, two other proteins implicated in peptidyltransferase activity (1, 7), and to L25, a member of the 5 S RNA complex (1, 9). Protein L16 also forms cross-links with 30 S proteins S12 and S19, indicating that L16 is near the subunit interface.

These results point to an important location for protein L16 at or near the peptidyltransferase center, the 5 S RNA, and the subunit interface. L16 also has an effect on the assembly and conformation of the 50 S subparticle and the transferase center, and it is not clear whether it contributes more directly to catalytic activity (10, 11). We have prepared a monoclonal antibody directed against protein L16. We report the effect of the antibody on functional properties of the 50 S ribosomal subunit as well as the first documented location of the protein epitope on the ribosome surface as determined by immunoelectron microscopy and discuss the correlation between these biochemical and microscopic results.

EXPERIMENTAL PROCEDURES AND RESULTS

Recognition of Ribosomes and Ribosomal Subunits by Anti-L16 Antibodies—The anti-L16 antibody was tested for its capacity to interact with 70 S ribosomes and their two subunits by enzyme-linked immunosorbent assay as shown in Fig. 1. Constant amounts of the ribosomal particles were titrated with increasing concentrations of the antibody. The anti-L16 antibody reacts strongly with 50 S particles, but not with 30 S or, surprisingly, 70 S particles.

Effect of Anti-L16 Monoclonal Antibody on Reassociation of Ribosomal Subunits—The failure of anti-L16 to react with the intact 70 S ribosomes suggested that the epitope was located at the interface, possibly shielded by the 30 S subunit. Protein-protein cross-linking experiments had shown cross-linking of L16 to two 30 S proteins, S12 and S19 (7, 8). These results prompted experiments to test the effect of the antibody on the reassociation of the two subunits. Reassociation of anti-L16-50 S complexes and 30 S subunits was measured by sucrose density gradient high speed centrifugation. The results are shown in Fig. 2. In buffer containing 10 mM Mg2+, a standard concentration for maintaining ribosome integrity and the stability and formation of control 70 S couples, both the antibody and the Fab fragments inhibited formation of 70 S ribosomes. When 50 S subunits were preincubated with 11 molar amounts of either anti-L16 or Fab fragments (Fig. 2, B and C), there was no detectable reassociation with 30 S subunits to form 70 S ribosomes. Fig. 2A shows the 70 S peak formed in the control experiment in the absence of the antibody. In buffers containing 15 mM Mg2+ (the concentration typically used for in vitro functional assay systems including polyphenylalanine synthesis), both the antibody and its Fab

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1 Portions of this paper (including "Experimental Procedures," part of "Results," and Fig. 9) are presented in miniprint at the end of the paper. The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography.
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FIG. 1. Recognition of ribosomes and ribosomal subunits by anti-L16 monoclonal antibody. Ten pmol of the 70 S ribosome and 50 S and 30 S ribosomal subunits were immobilized in the wells of microtiter plates, and their reactivity against the anti-L16 antibody (Ab) was determined by enzyme-linked immunosorbent assay using goat anti-mouse IgG coupled with horseradish peroxidase. O, 70 S ribosomes; ●, 50 S ribosomal subunits; □, 30 S ribosomal subunits.

Fig. 2. Effect of anti-L16 monoclonal antibody and Fab fragments on reassociation of ribosomal subunits. One A_{260} unit (40 pmol) of 50 S ribosomal subunits was preincubated with an equimolar amount of nonimmune mouse IgG (A), intact anti-L16 antibody (B), and Fab fragments (C) and then mixed with a 3-fold molar excess of 30 S subunits in buffer containing 10 mM Mg^{2+}. Formation of 70 S ribosomes was estimated by sucrose density gradient centrifugation. A similar experiment was done with the anti-L16 monoclonal antibody (D) and Fab fragments (E) in buffers containing 15 mM Mg^{2+}. Arrows mark the positions at which 70 S, 50 S, and 30 S particles sediment.

fragments did not show inhibition of subunit reassociation, even at 6-fold molar excess over 50 S subunits (Fig. 2, D and E). The antibody had no effect on 70 S ribosome dissociation, consistent with the enzyme-linked immunosorbent assay result (Fig. 1) that showed no binding to 70 S particles (data not shown).

Effect of Anti-L16 Monoclonal Antibody on Polyphenylalanine Synthesis—The antibody or its Fab fragments were incubated with 50 S subunits prior to the addition of 30 S subunits and initiation of the assay for protein synthesis. As shown in Fig. 3, both the antibody and the Fab fragments strongly inhibited polyphenylalanine synthesis. Almost 90% inhibition of polyphenylalanine synthesis occurred with amounts of the antibody or Fab fragments equimolar to ribosomes. Increased antibody concentration did not increase the inhibition. Nonimmune mouse IgG had no significant inhibition of polyphenylalanine synthesis in control experiments.

Effect of Anti-L16 on Peptidyltransferase Activity—L16 has been implicated as a component of the peptidyltransferase center. A distinctly different ribosomal activity promoted by a clearly separate ribosomal center is the binding of elongation factors. The effects of anti-L16 on both activities were tested. Anti-L16 had no effect on binding of elongation factors Tu and G or on their GTPase activities (data not shown). By contrast, as shown in Fig. 4, both anti-L16 and its Fab fragments were potent inhibitors of peptidyltransferase activity. Approximately 80% inhibition of N-acetylphenylalanyl-

FIG. 3. Effect of anti-L16 monoclonal antibody and Fab fragments on poly(U)-directed polyphenylalanine synthesis. The antibody (Ab) or its Fab fragments were incubated with 50 S subunits (40 pmol) at the molar ratios indicated prior to addition of 30 S subunits at the molar ratios indicated. The preincubated ribosomal subunits were then tested for their polyphenylalanine synthesis activity using [35S]phenylalanine. ●, intact L16 antibody; ○, Fab fragments; *, nonimmune mouse IgG.

FIG. 4. Effect of anti-L16 monoclonal antibody and Fab fragments on peptidyltransferase activity. Seventy-eight pmol of 50 S subunits were preincubated with different molar ratios of the antibody or Fab fragments, and peptidyltransferase activity was determined by measuring N-acetylphenylalanylpuromycin formation. ●, intact L16 antibody; ○, Fab fragments; *, nonimmune mouse IgG.
puromycin formation occurred at a molar ratio of one antibody/50 S subunit with either the intact antibody or its Fab fragments.

Epitope Mapping of L16—Protein L16 has a single histidine residue at position 13 (12). It has been shown that the modification of the histidine residue prior to reconstitution leads to the complete loss of peptidyltransferase activity in the resultant particles (10, 11). The possibility that the L16 epitope for the monoclonal antibody described here may include His13 was tested. Both pure L16 and 50 S ribosomal subunits were chemically modified with diethyl pyrocarbonate, and the reaction with anti-L16 was tested by immunoblotting following sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Fig. 5A shows pure L16 and the 50 S ribosomal proteins stained with Amido Black. A duplicate nitrocellulose sheet reacted with anti-L16 (Fig. 5B) showed no antibody reaction with modified pure L16 or with modified L16 from the mixture of total 50 S ribosomal proteins.

Localization of Binding Site by Immunoelectron Microscopy—Ribosomal subunits plus the monoclonal antibody were permitted to form complexes. Attempts to purify the complexes by size-exclusion high performance liquid chromatography were not successful; very few complexes survived the chromatographic separation even at ice bath temperatures. However, grids prepared from unfractionated reaction mixtures immediately after dilution showed many complexes involving either a single 50 S subunit plus one IgG molecule (monomeric complexes) or a pair of subunits linked by a single IgG (dimeric complexes).

A gallery of monomeric subunit complexes is shown in Fig. 6. In the most common quasi-symmetric view, antibody attachment is always seen to be beside and/or slightly below the subunit head (or central protuberance). In row 1, the subunit stalk projects at an angle; antibody contact is always on the side of the head distal to the stalk, and the Fab arm contacting the subunit usually appears slightly obscured. In row 2, the stalk projects more horizontally; again, contact is beside the head, on the side opposite the stalk. Many subunits lack the stalk and show 2-fold symmetry; antibody binding (row 3) is beside the head, in a position consistent with that described above. Occasionally, complexes are seen in which the subunit is in the asymmetric orientation; row 4 shows such images, and antibody contact is at the flat surface, near but not at the subunit head. Row 5 shows subunits in an intermediate projection in which the head appears at one side of the particle; antibody contact is seen at the base of the head, along the plane of the shoulders of the subunit.

Antibody-linked subunit dimers are shown in Fig. 7. All of the orientations of subunits described above are seen, and contact points with antibodies correspond to those seen in Fig. 6. No secondary site has been identified in either monomeric or dimeric complexes, and we have seen no clear instances in which more than one antibody molecule was complexed with a subunit. A total of 125 micrographs showing 4 x 104 subunits and complexes were evaluated for this work; 92% were consistent with a single location, shown in Fig. 8. The observations are quantified in Table I.

**DISCUSSION**

The results with the anti-L16 monoclonal antibody used in this work both confirm and help to explain further the major role attributed to L16 in the peptidyltransferase center. Both the intact antibody and its Fab fragments strongly inhibit peptidyltransferase activity at relatively low ratios of antibody
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### Table I

Numbers of antibody-subunit contacts seen in electron micrographs

<table>
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<th>Contact site</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Other</th>
<th>Total</th>
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<td>18</td>
<td>6</td>
<td>4</td>
<td>202</td>
<td>364</td>
</tr>
<tr>
<td>All views</td>
<td>642 (91.8)*</td>
<td>32 (4.6)</td>
<td>12 (1.7)</td>
<td>4 (0.6)</td>
<td>9 (1.3)</td>
<td>699</td>
</tr>
</tbody>
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* Numbers in parentheses indicate percentages.

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**Fig. 7.** Electron micrographs of antibody-directed dimeric subunit complexes. Subunits are shown in various orientations. Bar length: 50 nm.

**Fig. 8.** Localization of protein L16 on 50 S ribosomal subunit. The subunit is shown in the quasi-symmetric projection. Placement of other ribosomal proteins is based on results from our laboratories using monoclonal antibodies and immunoelectron microscopy as probes. L16 at the peptidyltransferase center, exactly as predicted by the biochemical results. Fig. 8 depicts the location of L16 in the quasi-symmetric view of the 50 S subunit beside the central protuberance of the 50 S particle on the side away from the L7/L12 stalk and on or near the interface between the two subunits. Fig. 8 also summarizes the locations of other ribosomal proteins studied in our laboratories using monoclonal antibodies and immunoelectron microscopy as probes. The protein is very close to our placements of proteins L5 (13) and L2 (14); monoclonal antibodies against these proteins inhibited peptidyltransferase activity. Protein L2 was studied with two monoclonal antibodies, with epitopes within residues 5–186 and 187–272, designated N and C in Fig. 8, respectively. Although both antibodies strongly inhibited peptidyltransferase activity (15), the N-terminal epitope appears nearer the L16 epitope identified. On the other hand, mutations in the C-terminal region of L2 between residues 221 and 231 destabilize the binding of L16 (16), a result that suggests that L16 may extend beyond the location indicated by the single monoclonal antibody used here. The location of the L16 epitope is also near the site of 50 S subunit photoaffinity labeling by puromycin (17) or azidopuromycin (18), both of which serve to localize the peptidyltransferase center. The site is consistent with cross-links between L16 and proteins L18 (a 5 S RNA-binding protein in the central protuberance (9)) and L23 (the primary component labeled by puromycin (19) or azidopuromycin (20)). Our placement of L16 is close but not identical to a localization from the laboratory of Stoffler and Stoffler-Meilicke (21). Since a later publication from the same group (22) indicates that their placement of L16 is based on cross-linking data (in Table I of Ref. 22) and not on immunoelectron microscopy, it appears that the work presented here represents the first direct documentation of the location of L16 by immunoelectron microscopy.

The magnesium dependence of the accessibility of the antibody to the ribosome supports the conclusion from cross-linking that L16 is near the 50 S–30 S subunit interface. The antibody binds to free 50 S subunits, but not to preformed 70 S couples. Antibody–50 S subunit complexes fail to associate at 10 mM Mg2+, but the antibody or its Fab fragments are not an absolute block and can be overcome by raising the level of Mg2+ to 15 mM. (Hence, the inhibition of polyphenylalanine synthesis under the conditions of the protein synthesis assay is not due to the failure of antibody–50 S subunit complexes to form 70 S ribosomes.) Studies on reconstitution (23, 24) and particles lacking L16 (16) show that protein L16 is associated with a conformational change within the peptidyltransfase center (3). Subunit association appears to be a cooperative process in which both RNA and protein are

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involved; our results suggest that antibody binding to protein L16 partially blocks this association and perturbs one part of the subunit interface. However, under some conditions, association can occur, but the blocking or distortion of the transferase center is retained. Our placement of L16 by immunoelectron microscopy is compatible with these conclusions and with the interface cross-link of L16 to protein S19 (6–8), a tRNA-binding protein of the small subunit (26) that is located opposite the transferase center in 70 S ribosomes (27).

The epitope of protein L16 with which this monoclonal antibody interacts has not been mapped precisely, but treatment of L16 with a histidine-specific reagent, diethyl pyrocarbonate, destroys the interaction with the monoclonal antibody. The reaction of this reagent with the single histidine (10, 11) and altered interaction of L16 with the 50 S subunit (3). The N-terminal fragment of protein L16 (residues 1–47 (28) and, in particular, residues 10–16 (29)) has been implicated as essential for the restoration of peptidyltransferase activity to depleted cores. These observations, in conjunction with inhibition of peptidyltransferase activity by the anti-L16 antibody, are all explainable by postulating an interaction of the His45 region of L16 either directly with peptidyltransferase reactants or with other catalytic components as well as with the antibody. Alternatively, the His45 region of L16 could function in maintaining the conformation of the entire protein since evidence suggests its participation in higher order structure. Littlechild et al. (30) have shown that isolated L16 has significant secondary structure, and the results of chemical modification experiments indicate that the N-terminal portion is relatively less reactive and so is presumably more structured (28). Histidine modification may alter L16 conformation so as to perturb both transferase activity and antibody recognition. The epitope recognized by this anti-L16 monoclonal antibody is clearly near the peptidyltransferase center. Other antibodies may reveal regions of L16 involved in its assembly and interaction with L2.

REFERENCES
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EXPERIMENTAL PROCEDURES

Preparation of ribosomes, ribosomal subunits and 5-100 enzymes

Tight coupling of ribosomes from E. coli MRE600 cells were prepared from slowly cooled mid-log cultures as described earlier. Ribosomal subunits were recovered by anonal centration at 1 mM MgCl₂ (31). The first high speed supernatant during the ribosome isolation procedure was used as S1000 and concentrated to 8 fold by vacuum dialysis against 10 mM Tris-HCl pH 7.4, 10 mM MgCl₂, 100 mM NH₄Cl and 14 mM 2-mercaptoethanol.

Purification of ribosomal protein L16

Total ribosomal proteins were extracted from 50S subunits with 67% acetic acid as described by Hendy et al (32). Dialyzed against 6% acetic acid and kholinated. The proteins were dissolved at a concentration of 8 mg/ml in buffer containing 6 M urea, 0.05 M Na₂HPO₄, pH 6.5, 12 mM methylmercury and 4 mM 2-mercaptoethanol and loaded on phosphocellulose column (50 cm x 1.3 cm) which had been washed and equilibrated with the same buffer (133). The flow rate was maintained at 18 ml/hr and the column was washed with 2 bed volumes of the starting buffer. The protein were then eluted with a linear gradient of 0-0.5 M NaCl in 3 liters of the same buffer. Fractions of 9 ml were collected. The eluant was monitored at 280 nm and protein peaks were analysed by one-dimensional SDS-PAGE (34). Fractions containing L16 were combined, dialyzed against 4% acetic acid and lyophilized. The L16 thus obtained was contaminated with L2 and was purified to homogeneity by HPLC on an HPSEC-C3 column (Alltech) with a gradient of acetonitrile in 0.1% TFA as solvents (29).

Hydroloma production, cloning and ascites preparation

Eight-week-old BALB/c mice were inoculated with HPLC purified protein L16. Antibodies in the hydroloma culture fluid were detected by standard enzyme ELISA using 10 fold of either 50S ribosomal subunit or purified L16 as antigen to coat microtiter plates (36). Hydroloma cultures specific for L16 were selected on four times by limiting dilution (37-28) using thymocytes and macrophages as feeder cells. Ascites fluid was prepared in pristane primed mice, tappled and clarified and described earlier (38) and stored at -80°C.

Purification of antibodies from ascites

L16 specific monoclonal antibody was purified from ascites fluid in one step by affinity chromatography using partially purified L16 proteins coupled to Affigel 10 as the solid phase support as described previously (39). The purified antibody was concentrated to about 20 mg by vacuum dialysis against 10 mM Tris-HCl, pH 7.2, 1 mM magnesium acetate and 150 mM NH₄Cl and stored at -80°C.

Preparation and purification of monoclonal Fab fragments

Fab fragments were purified from affinity purified antibodies by papain digestion as described previously (29). The Fab fragments were purified by affinity chromatography and analyzed as described above.

Immunoglubulin subtype and light chain identification

Subtyping of immunoglobinulins and light chain identification was carried out using subclass specific (lymphoma antibodies. The procedure described by Ouriander Mannheim. Biochemica, supplier of the subclass identification kit, was followed.

Immunohistochemistry

Total protein of 50S and 30S ribosomal subunits were electrophoresed in a 5% polyacrylamide gel, transferred to a nitrocellulose membrane and analyzed for reactivity with antibody to L16 as described earlier (40).

Poly U-directed polyphenylalanine synthesis

The poly U directed polyphenylalanine synthesis was as described earlier (29-30). Forty pmol of 50 S ribosomal subunits were incubated for 15 min at 37°C with different amounts of L16 antibodies prior to the addition of 30 S subunits.

Association of ribosomal subunits

One A260 unit (40 pmol) of 50 S subunits was preincubated with different amounts of antibody or Fab fragment at 37°C for 15 min in a total volume of 200 µl of 10 mM Tris HCl, pH 7.2, 100 mM NH₄Cl, 7 mM 2-mercaptoethanol and either 10 mM or 15 mM MgCl₂. A three fold molar excess of 30 S subunits was added, and the mixture was kept at 0°C for 30 min. Each sample was then layered on a 7.5-25% linear sucrose gradient. Centrifugation was at 4°C for 80 min at 55,000 rpm in a Beckman SW4 O6 rotor and the gradient was analyzed using a Gilford spectrophotometer equipped with a flow cell to monitor absorptions at 280 nm.

Preparation of RNA and N-acetyl-4-thioRNA

RNA from E. coli was prepared according to the method of Zulon (41) and was charged N-acetyl-4-thioRNA as described by Sorm (42). N-acetyl-4-thio-phenylalanine RNA was prepared as described by Hennig and Chepilve (43).

Peptidase transferase activity assay

The assay was of Hamp et al (40) was used with some modifications. A typical assay was done with 76 pmol of 50 S subunits that were preincubated at 37°C for 10 min with different molar amount of antibodies or Fab fragments in a total volume of 200 µl buffer containing 20 mM Tris HCl pH 7.8, 150 mM NaCl and 15 mM magnesium acetate. The remaining components of the assay were then added to give a final volume of 500 µl containing 30 mM Tris HCl, pH 7.8, 150 mM NaCl, 250 mM KC1 and 15 mM magnesium acetate. 0.3 µl pyruvate and 40 pmol of 4-thio N-acetyl-4-thioRNA. The reaction mixture was incubated at 37°C for 10 min and the reaction was stopped by the addition of 100 µl of 0.3 M sodium acetate pH 5.5, saturated with MgCl₂, 2.5 mM dithiothreitol. The reaction was added and the mixture was shaken vigorously for 1 min and allowed to stand for 5 min. After phase separation, the upper 2 ml were withdrawn, mixed with 5 ml of sodium silicate fluid (Aquaman, Westcistem) and (0.5) was measured in a liquid scintillation counter.

The aminoacyl thiopeptide CACCA-lacT(C8-D) fragment was prepared and purified as described (44). The fragment reaction was assayed according to Moris (45) with the following modifications. 30 pmol of 50 S ribosomal subunits were preincubated for 10 min at 37°C with different amounts of antibodies in 30 µl buffer containing 10 mM Tris HCl, pH 7.6, 2 mM MgCl₂ and 150 mM HCl. After preincubation the remaining components of the assay were added to give a final reaction volume of 500 µl containing 30 mM Tris HCl, pH 7.6, 150 mM NaCl, 250 mM KC1, 20 mM magnesium acetate, 6.000 cm of CACCA-lacT(C8-D) fragment (specific activity 500 cpm/mkoulb, 4 mM magnesium and 35% methanol. After incubation for 10 min at 0°C, the reaction was stopped by addition of 100 µl of 0.3 M sodium acetate, pH 5.5, saturated with MgCl₂, 1.5 ml of ethyl acetate was added, the mixture was agitated for 30 sec and centrifuged at 1000 x g, 1 ml of the upper layer was mixed with 3 ml of scintillation fluid and radioactivity was measured.

Modification of ribosomes and L16 proteins with dithiyl pyrocarbonate

The procedures were based on those described by Tate, et al (46). Reaction mixture for L16 included: 480 pmol of purified L16 protein in 20 mM Tris HCl pH 7.6, 4 mM magnesium acetate, 400 mM NaCl, 4 mM 2-mercaptoethanol, 0.2 mM EDTA, 30% v/v ethanol, and 30 mM dithiylpyrocarbonate; and for 50 S subunits: 100 pmol 50S ribosomal subunits in 20 mM Tris HCl pH 7.6; 60 mM NaCl, 10 mM magnesium acetate, 10 mM 2-mercaptoethanol, 0.1% ethanol, and 30 mM dithiylpyrocarbonate. The reaction mixtures were incubated at 25°C for 30 min and the reaction was stopped by adding sodium carbonate at a molar excess over dithiylpyrocarbonate followed by incisionation for 10 min. Modified L16 was dialyzed against water and lyophilized. Modified ribosomal subunits were dissolved against the reaction buffer and the proteins were extracted as described earlier above.

Electron microscopy

Ribosomal 50 S subunits (2 x 15 pmol) plus 9-20 pmol of antibody were incubated for 5 min at 37°C and then overnight on ice in 30-50 µl of 20 mM Tris HCl pH 7.5, 100 mM NaCl, 10 mM magnesium acetate buffer. In some instances the reaction mixtures were subjected to sucrose elusion by using a Beckman/Alfors TSK 3000 column (7.5 x 150 cm) and the samples buffer at a flow rate of 1 ml/min. Buffers and columns were packed in ice. Alternatively, the reaction mixtures were simply diluted to 1 ml for grid preparation. Samples were absorbed to thin carbon films and negatively stained with 1% uranyl acetate as described by Lake (47). Electron micrographs were obtained using a JEOL 1200 FX electron microscope operated at 80kV and a magnification of 100,000.

RESULTS

Specificity of the monoclonal antibody for L16

A hybridoma cell line secreting monoclonal antibody against E. coli protein L16 was produced by immunizing mice with HPLC-purified L16 protein. The antibody was purified from affinity chromatography using partially purified L16 antibodies and analyzed as described above. Antibody specificity was evaluated by immunostaining from one and two dimensional gels of proteins from both 50 S and 30 S ribosomal subunits. One dimensional SDS PAGE shows that the antibody reacts only with a single component of 50 S subunits in a region where protein L16 migrates (Fig 4). The specificity of the antibody for L16 was shown by a two dimensional PAGE system containing SDS in the second dimension in which L16 is clearly resolved as a single spot.