THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 246, No. 14, Issue of July 25, pp. 4504-4509, 1971
Printed in U.S.A.

Reversible Denaturation of Enzymes by Sodium Dodecyl Sulfate*

(Received for publication, April 8, 1971)

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SUMMARY

Successful renaturation of several oligomeric enzymes after denaturation by sodium dodecyl sulfate is described. The results obtained suggest a general procedure for reactivation of enzymes after treatment with this detergent. Proteins in dodecyl sulfate solution are first incubated in concentrated urea and are then freed of the detergent by an anion exchange resin. The resulting dodecyl sulfate-free proteins can be renatured from urea solution by standard procedures. This method has also been used to recover enzymatic activity in moderate yields of some proteins after electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulfate.

Exposure to sodium dodecyl sulfate causes denaturation of proteins and dissociation of oligomeric structures. Recent studies have shown, that a variety of proteins bind identical amounts of SDS[1] on a weight to weight basis. Above a SDS-monomer concentration of 8×10^{-4} M, 1 g of protein binds 1.4 g of SDS (1, 2). Studies of the hydrodynamic and optical properties of the resulting protein-SDS complexes indicate rodlike particles in which the molecular length varies uniquely with the molecular weight of the polypeptide chain (3, 4). This general mechanism for SDS-protein interaction explains why the electrophoretic mobilities of numerous polypeptide chains upon electrophoresis in SDS-polyacrylamide gels are found to be solely dependent on their molecular weights (5, 6).

Denaturing agents like urea or guanidine hydrochloride transform proteins into an random coil conformation (7) and the removal of these agents is generally accompanied by recovery of the native structure (8, 9). However, renaturation of proteins from SDS, which tightly binds to proteins, has thus far not been obtained. Given the ability of proteins to regain their native conformation in an appropriate renaturing environment, we have studied the conditions for renaturation after exposure to SDS of several oligomeric proteins. Preliminary results on the successful reconstitution of aspartate transcarbamylase (10) led us to a general procedure for the renaturation of enzymes from SDS.

MATERIALS AND METHODS

Chemicals—Dowex AG 1-X2 (200 to 400 mesh) was obtained from Bio-Rad (Richmond, California). It was exhaustively washed on a Buchner funnel with the following solvents: 2 N NaOH, distilled water, 4 N acetic acid, distilled water, and finally 0.05 M Tris-acetate buffer, pH 7.8. The resin was stored in this solution for several weeks. Prior to use a small amount of resin was equilibrated in a centrifuge tube with Buffer A (0.05 M Tris-acetate, pH 7.8, 0.01 M 2-mercaptoethanol), 6 M in urea, if not otherwise specified.

Reagents for polyacrylamide gel electrophoresis have been described previously (6) with the following exception. We have routinely used high purity grade acrylamide (Bio-Rad) and sodium dodecyl sulfate ("Sequenal grade," Pierce Chemical Company, Rockford, Illinois) without recrystallization.

Sephadex G-200 was obtained from Pharmacia. Urea was purchased from the R Plus Company (Denville, New Jersey) and used without recrystallization. Urea solutions were freshly made prior to use. ^{35}S-sodium dodecyl sulfate was obtained from Amersham-Searle, Des Plaines, Illinois (11.8 mCi per mM). All chemicals used were reagent grade if not otherwise specified.

Proteins—Aspartate transcarbamylase from *Escherichia coli* and its catalytic subunit were isolated as described by Gerhart and Holoubek (11) and assayed as previously reported (10). RNA-polymerase from *E. coli* was purified by the procedure of Berg, Barrett, and Chamberlain (12) and assayed as reported by Burgess *et al.* (13). *Lac* repressor of *E. coli* was a gift of T. Platt and had been purified by the procedure of Gilbert and Mueller-Hill (14). The inducer-binding activity for isopropyl-1-thio-β-D-galactopyranoside was assayed by the Millipore assay of Riggs and Burgeois (15). β-Galactosidase from *E. coli* was available as a partially purified preparation and as crystallized enzyme (gift from Dr. R. Weil). The enzyme was assayed as described by Paigen (16). Rabbit muscle aldolase was the commercially available crystalline product from Worthington and was assayed as described elsewhere (17). R17 coat protein was prepared from *E. coli* bacteriophage R17 as described previously (18). Bovine serum albumin (Fraction V, B grade) was obtained from Calbiochem.

Removal of SDS by Dowex 1-X2 in Buffered Urea Solution—In these experiments proteins at a concentration of 2 mg per ml were incubated for at least 1 hour in 1% SDS at room temperature. When higher protein concentrations were used, SDS was always present in at least a 5-fold by weight excess to assure complete dissociation. To avoid disulfide formation, all solutions were maintained at 0.01 M in 2-mercaptoethanol. The hydrodynamic studies of Reynolds and Tanford (3) as well as earlier sedimentation studies (19, 20) show that dissociation is complete under these conditions. After this treatment, the protein solutions were made 6 M in urea either by adding solid urea and incubating for 30 min or by dialyzing for 1 hour at room temperature against

* This work was supported by Grant GM-16132 of the National Institutes of Health.

[1] The abbreviation used is: SDS, sodium dodecyl sulfate.

Buffer A (0.05 M Tris-acetate, pH 7.8, 0.01 M 2-mercaptoethanol), 6 M in urea. The SDS was removed by passing the protein solutions through a small column (1 cm diameter, 10 to 15 cm length) of Dowex 1-X2 equilibrated with Buffer A, 6 M in urea. The column was developed with the same solvent. Protein-containing fractions of the effluent were pooled. Such a column, packed with the resin and run at a rate of 0.5 ml per min, binds at least 100 mg of SDS for every milliliter of the settled resin.

An alternative procedure was used with very small volumes containing SDS. A thick suspension of Dowex 1-X2 was directly added into the protein-SDS-urea solution. After incubating for 5 to 30 min, the resin was removed by passing the suspension through a 1-ml Tuberculin syringe equipped with either a very fine needle or a needle plugged with a small amount of glass wool. Application of gentle pressure with the plunger allows the solution to pass through the needle while the resin is retained.

Reconstitution from Urea Solution—Proteins were usually reconstituted at room temperature by dropwise dilution into Buffer B (0.05 M Tris-acetate, pH 7.8, 0.01 M 2-mercaptoethanol, 0.1 mM EDTA) so that the final dilution was at least 10-fold. Any insoluble material appearing during dilution was immediately removed by centrifugation. Such precipitation was often avoided by adding bovine serum albumin to Buffer B at a concentration of 0.1 mg per ml. The solutions were concentrated by ultrafiltration in a small Amicon cell (UM10 membrane) and finally dialyzed against Buffer B to remove residual urea. All operations after dilution were carried out at 4°. Protein concentration was determined by the Lowry method or by optical density at 280 nm. Standard enzymatic assays were performed in triplicate.

Aldolase was optimally reconstituted from urea by dilution as above or by dialysis with the procedure of Stellwagen and Schachman (21). Enhanced yields of β-galactosidase were obtained by following the method of Ullmann and Monod (22). Although *lac* repressor was optimally reactivated from Buffer B, the reactivated protein was more stable when diluted into Buffer B in which 1 M Tris-HCl substituted for 0.05 M Tris-acetate. RNA-polymerase (0.3 mg) was treated in 0.5 ml of 0.02 M Tris-HCl, pH 8, with SDS at a concentration of 0.3%. After 3 hours, the solution was dialyzed for 1 hour at room temperature against Buffer C (0.5 M Tris-acetate, pH 8, 0.01 M magnesium acetate, 0.02 M 2-mercaptoethanol, 1 mM EDTA, 6 M urea) 20% in glycerol, and 0.1% in SDS. Dowex 1-X2 was equilibrated with Buffer C. After treatment with this resin the SDS-free protein was dialyzed against Buffer C, 20% in glycerol, for 1 hour at room temperature, followed by overnight dialysis in the cold against a large volume of 0.05 M Tris-acetate, pH 8 (0.01 M magnesium acetate, 1 mM EDTA, 0.15 M NH_4Cl, 20% glycerol, 0.02 M 2-mercaptoethanol). After dialysis the sample was kept for 45 min at room temperature and then cooled to 4°. Enzymatic activity was determined after the final incubation at room temperature (23).

Polyacrylamide Gel Electrophoresis in 0.1% SDS—Gel electrophoresis on 5 and 10% acrylamide gels was performed essentially as described by Weber and Osborn (6). Gels used for recovery of protein were routinely prerun with a low molecular weight protein. R17 coat protein (molecular weight 13,800) was dissolved at a concentration of 2.0 mg per ml in 1% SDS, 1% 2-mercaptoethanol, 0.01 M sodium phosphate buffer, pH 7.0, and then dialyzed against 0.1% SDS, 0.1% 2-mercaptoethanol in the same buffer. Each gel was loaded with 50 to 100 μg of protein in this solution. Electrophoresis was performed at 4 ma per tube for 3 hours longer than the period required for the dye (bromphenol blue) to reach the bottom of the gel. Staining of such gels revealed no residual protein material. To avoid changes in pH of the running buffer the buffer in the reservoirs was changed twice during the electrophoresis.

Gels were also prerun with 50 μl of a solution containing 0.01% crystal violet and 0.1% SDS in 0.01 M sodium phosphate buffer, pH 7. Under these conditions the dye migrated to the anode, probably because of complex formation with dodecyl sulfate anions. "Preparative gels" were run at 3 ma per tube. After electrophoresis the gels were sliced into four longitudinal pieces with the slicing device described by Fairbanks, Levinthal, and Reeder (24). While three strips were kept in Saran Wrap to prevent drying, the fourth was stained for 15 min in Coomassie brilliant blue staining solution and destained by diffusion in destaining solution (6). The protein bands became visible after 60 to 90 min. The corresponding pieces of the three other gel strips were cut out, transferred into test tubes, and covered with 0.1% SDS solution. Up to 0.5 ml of solution was used. Incubation was performed for 6 to 12 hours at room temperature. The eluate was then dialyzed for 1 hour against Buffer A, 6 M in urea, and treated with Dowex 1-X2 as described above for small sample volumes. After removal of SDS the urea solution was made 0.01 M in 2-mercaptoethanol and incubated for 2 hours at room temperature before reconstitution.

Gel Filtration on Sephadex G-200 in 1.0% SDS—Sephadex G-200 was equilibrated in 1% SDS, 0.01 M NH_4HCO_3, and a column (70 × 3 cm) was filled with the resin. The column was standardized with proteins of well characterized polypeptide chain molecular weights (4, 10). Of a 10% SDS solution, 0.5 ml was added to 1 ml of aspartate transcarbamylase solution (10 mg per ml) in 0.1 M Tris-acetate, pH 7.5, and the mixture dialyzed for 8 hours against 1% SDS. Of the protein solution, 1.5 ml were applied to the G-200 column. As reported elsewhere (10), two protein peaks corresponding to the catalytic and regulatory polypeptide chains of this enzyme were found. Their K_D values corresponded to the known molecular weights of these polypeptide chains. Protein-containing fractions were pooled and lyophilized. The dry powder was dissolved in 10% of its original volume, dialyzed against Buffer A, 6 M in urea, and incubated for several hours to assure complete reduction of all cysteine residues. The solution was passed through a 10-ml column of Dowex 1-X2, equilibrated, and developed as described above.

Sedimentation Velocity Analysis—Sedimentation coefficients of renatured enzymes were obtained by centrifugation in a Spinco model E ultracentrifuge equipped with schlieren optics. All runs were performed at 20° in a cell with 30-mm path length with an AN-B-E rotor at a speed of 50,740 rpm. The protein concentrations were approximately 1.4 mg per ml. Sedimentation coefficients were calculated and compared with the values obtained in parallel runs with native proteins. Buffer B was used in all experiments if not otherwise specified. The sedimentation coefficient of renatured β-galactosidase was obtained by sucrose gradient centrifugation (25) with native β-galactosidase and bovine catalase as marker proteins in parallel gradients.

Fingerprint Analysis of Proteins Eluted from SDS Gels—Protein bands are localized (see above) and corresponding gel slices eluted with 0.1% SDS in 0.05 M NH_4HCO_3. The eluates are concentrated by lyophilization and redissolved in a small volume

of 6 M urea, 0.05 M NH_4HCO_3. SDS is removed by treatment with Dowex 1-X2 equilibrated with the same solvent. Urea and salt can be removed by dialysis against water. Tryptic digestion can also be performed on the protein in urea solution after 3-fold dilution with water. The tryptic digest is applied to a small column of Dowex 50-X2 (H^+ form) and the urea removed by washing with distilled water. The peptides are then stripped from the resin with 2 M NH_4OH and the ammonia removed from the effluent by lyophilization. For very small amounts of radioactively labeled proteins addition of a carrier protein after elution from the SDS gel is advisable.

RESULTS

Removal of SDS—Earlier attempts to remove SDS from protein solutions by prolonged dialysis have given ambiguous results. Putnam and Neurath reported incomplete removal by dialysis and suggested precipitation with barium salts (26). However proteins thus recovered showed increased electrophoretic mobilities indicating residual SDS binding. Furthermore McMeekin *et al.* noticed that the precipitation procedure was not successful since they obtained a stable complex of 2 molecules of SDS per molecule of β-lactoglobulin from which they were unable to dissociate the detergent by dialysis or treatment with barium hydroxide (27). Pitt-Rivers and Impiombato have reported precipitation of some proteins upon dialysis to remove the SDS (1). With the exception of ribonuclease, more recent attempts to regain native protein structures by dialysis from SDS solutions have also failed (28). The poor results obtained by dialysis suggested that an alternative procedure should be found to free proteins of the detergent. Earlier work of Stark, Stein, and Moore (29) had indicated that SDS might be removed from proteins by ion exchange chromatography. In their experiments pancreatic ribonuclease in SDS solution was applied to a column of Dowex 1 and the eluted protein was found to be enzymatically active. However ribonuclease is not an ideal protein with which to illustrate the reversibility of binding of SDS. Several experiments have shown that it binds SDS poorly because of the presence of disulfide bonds which inhibit denaturation by SDS (1–3). Consequently we attempted to remove SDS from different proteins by subjecting them in 6 M urea solution to the anion exchange resin Dowex 1-X2. Urea was used to avoid the possible precipitation of proteins upon removal of SDS, and to prevent their irreversible binding to the ion exchange resin. Presumably urea also may serve to weaken the SDS-protein interactions and so enhance the removal of SDS.

By use of ^{35}S-labeled SDS it was found that 1 ml of settled Dowex 1-X2 resin can easily bind 100 mg of SDS within less than 10 min. Although this number is lower than the theoretical capacity of the resin (approximately 300 mg), no attempts were made to use the full resin capacity. Next we studied whether all SDS molecules bound to a protein molecule can be stripped on passing the solution through the anion exchange column. In a typical experiment, 1 ml of a 5 mg per ml solution of aspartate transcarbamylase containing 35 mg of ^{35}S-labeled SDS (2×10^7 cpm total) in Buffer B was made 6 M in urea by the addition of solid urea. The solution was passed through a 0.6-ml column of Dowex 1-X2 previously equilibrated with buffer. The effluent was monitored for protein concentration and radioactivity. Nearly quantitative recovery of protein (95%) and no detectable radioactivity was found. Identical results were obtained with *lac* repressor and aldolase. These experiments indicate that at most only 1% of all the protein molecules will still bind 1 molecule of SDS.

Reactivation after Denaturation in SDS Solution—The efficient removal of SDS and excellent recovery of the protein after treatment with the ion exchange resin suggested a two-step procedure for the renaturation of proteins after dissociation and denaturation by SDS. In the first step the protein-bound SDS is removed by Dowex 1-X2 in urea solution yielding an SDS-free protein. The second step, the renaturation of the protein from urea, is considered to be a general property of proteins since numerous enzymes have been reactivated from urea or guanidine-HCl solutions (for a recent discussion see (30)). The results obtained with such a two-step renaturation process on six different oligomeric proteins[2] are shown in Table I.

We have renatured the proteins from urea simply by diluting the solution 10-fold, concentrating by ultrafiltration, and removing the residual urea by dialysis. With the exception of RNA-polymerase which was renatured only during dialysis, all proteins tested could be reactivated by dilution into Buffer B, although optimal reactivation often demanded more specific renaturation conditions. Thus in the case of aldolase only 20% reactivation was obtained by dilution into Buffer B and high reactivation (50%) was achieved only after a change in the pH of this buffer from 7.8 to 5.5.

Although we made no further attempts to optimize these recoveries for the urea renaturation step, it is very likely that with certain proteins better results can be obtained if a careful study of parameters like pH, temperature, ionic strength, salt ions, protein concentration, and buffer type is performed. The recovery of enzymatic activity is generally in good agreement with the values reported in the literature for recovery after renaturation from guanidine-HCl or urea. The yields reported in Table I range from 40 to 80% with RNA-polymerase as an exception, where only 15% reconstitution was obtained. However, Heil and Zillig (23) as well as Lill and Hartmann (34) pointed out that the renaturation recovery of this enzyme can vary. The low value obtained in this study is in the range reported by these authors.

Prolonged treatment with SDS as is necessary during gel filtration on G-200 or agarose (4, 10) does not interfere with the possibility of recovering enzymatic activity. Aspartate transcarbamylase was dissociated in 10% SDS at a protein concentration of 10 mg per ml and dialyzed for 6 hours against 1% SDS. One milliliter of the resulting solution was passed through a G-200 column in 1% SDS. The fractions containing the cata-

[2] The oligomeric enzymes used in this study vary in complexity and size of their polypeptide chains. *Lac* repressor and β-galactosidase are tetrameric molecules with identical polypeptide chains of molecular weights 38,000 (14) and 135,000 (31), respectively. The catalytic subunit of aspartate transcarbamylase contains three identical polypeptide chains of molecular weight 33,500. Aspartate transcarbamylase in its native form consists of six regulatory and six catalytic polypeptide chains of molecular weights 17,000 and 33,500, respectively (10, 32). The tetrameric aldolase molecule is assumed to contain two very similar polypeptide chains (33). RNA-polymerase contains at least five different polypeptide chains with molecular weights 160,000 (β′), 150,000 (β), 95,000 (σ), 41,000 (α), and 10,000 (ω), and most likely has the structure, $\beta'\beta\sigma\alpha_2\omega_2$ (13). The specificity polypeptide chain σ can be assayed by its stimulatory activity on the catalytic complex $\beta\beta'\alpha_2\omega_2$, with *E. coli* bacteriophage T4 as the DNA template (13).

TABLE I

Reactivation of enzymes after dissociation in SDS solution[a]

Protein	Used	Exposure to SDS	Recovery	Published recovery after urea or guanidine-HCl treatment
	mg	*hrs*	%	%
Aspartate transcarbamylase	1.8	1	46	60–70 (10)
	3.4	1	53	
	1.8	3	70	
	1.8	70	20	
	1.8	1	92[b]	
β-Galactosidase	3.0	5	65	100 (22)
	2.0	1	60	
	0.5	2	62	
Aldolase	1.7	2	20	50 (21)
	3.2	1	22	
	3.2	5	52[c]	
	5.0	2	30[d]	
Lac repressor	1.4	1	50	50–60[e]
	1.8	1	23	
	1.8	1	62	
	6.0	1	51	
	6.0	1	54	
RNA-polymerase	0.3	4	10	5–55 (23, 34)
	0.3	4	15[f]	
Catalytic subunit of Aspartate transcarbamylase	2.0	10	40	60 (10)
	4.0	5	35	
	0.8	5	40	
	2.0	2	45	

[a] Unless otherwise indicated, all proteins were treated as described under "Materials and Methods."

[b] Reconstitution by dilution into Buffer B in the presence of 0.1 mg per ml bovine serum albumin. The sample had been concentrated by lyophilization before treatment with urea.

[c] After removal of SDS, the enzyme-urea solution was diluted 10-fold into a buffer identical with Buffer B but adjusted to pH 5.5.

[d] Reconstitution from urea was performed according to Stellwagen and Schachman (21).

[e] Determined after renaturation from 6 M urea.

[f] In this experiment the dissociation buffer was 0.04 M in 2-mercaptoethanol rather than the usual 0.02 M.

lytic polypeptide chain were pooled (10). After lyophilization, removal of SDS, and renaturation, the recovery of enzymatic activity was approximately 40%. SDS treatment for periods longer than 50 hours seemed to result in somewhat decreased reactivation yields. In one case exposure of aspartate transcarbamylase to 1% SDS for 70 hours (Table I) resulted in only 20% reactivation in comparison with the 60 to 70% usually obtained upon incubation for less than 24 hours.

Since recovery of enzymatic activity by renaturation from urea is generally accompanied by restoration of the native tertiary and quaternary structure (8), we have subjected three of the renatured proteins to sedimentation velocity analysis. The reconstituted enzymes (β-galactosidase, aldolase, and aspartate transcarbamylase) showed only one predominant species which

TABLE II

Reactivation of enzymes from SDS-polyacrylamide gels[a]

Protein	Applied to gel	Recovery
	μg	%
Aspartate transcarbamylase (catalytic subunit)		
Experiment 1	280	36
	150	13
	55	16
	20	46
Experiment 2	280	25
	140	55
	70	50
	28	67
Experiment 3[b]	250	40
	150	20
	50	25
	20	60
Experiment 4	140	24
	140	29
	140	22
	140	14
Experiment 5[c]	140	11
	140	19
	140	14
Aldolase		
Experiment 1	200	15
	100	19
	35	25
Lac repressor		
Experiment 1	450	11
	270	25
Experiment 2[d]	450	4
	360	5
	90	15
σ factor of RNA-polymerase[e]		
Experiment 1	100	60
Experiment 2	40	50

[a] Unless otherwise stated gels were run and treated as described under "Materials and Methods." The recovery was corrected for the material lost by cutting a guide strip for the localization of the protein band on the gel. Yields were calculated from the recovery of the enzymatic activity.

[b] Reconstitution was carried out in the presence of 0.5 mg per ml of bovine serum albumin in Buffer B.

[c] Gels were prerun with crystal violet rather than R17 coat protein.

[d] Renatured by dialysis against Buffer B rather than by dilution.

[e] RNA-polymerase was applied to the gels and the protein corresponding to σ (approximately 20% of the applied enzyme (13)) was isolated. The σ protein was eluted from the gel into 1 mM dithiothreitol, 0.1 mM EDTA, 0.1% SDS, and then dialyzed against Buffer A, 6 M in urea. After removal of SDS the solution was diluted into Buffer B containing 0.25 mg per ml of bovine serum albumin and concentrated by dry Sephadex G-200.

sedimented with a value characteristic of the native protein.[3] Further evidence that the conformation of the renatured protein was similar to that of the native molecules was obtained by examining the kinetic properties of aspartate transcarbamylase. This enzyme in its native state contains six regulatory and six catalytic polypeptide chains (10) and is subject to allosteric inhibition by cytidine triphosphate which binds at the regulatory sites. Feedback inhibition by CTP was present in the renatured enzyme at a level 85% of that typical of native aspartate transcarbamylase, thus indicating that the two different types of polypeptide chains had correctly reassembled to form the full enzyme. Attempts have not been made to optimize the reconstitution conditions and to obtain material with better kinetic properties.

Attempts to Recover Enzymatic Activity after SDS Gel Electrophoresis—Since the procedure allows the reconstitution of very small quantities of proteins (20 to 50 μg) with small amounts of Dowex resin to remove the SDS, it seemed possible to attempt reconstitution of proteins separated on and eluted from SDS-polyacrylamide gels. Protein bands were localized by cutting a longitudinal guide strip and subjecting it to staining and destaining. Alternatively, a parallel gel was run and processed through the staining procedure. First attempts to reconstitute proteins eluted from polyacrylamide gels failed and yielded enzymatically inactive SDS-free protein after the ion exchange treatment and removal of urea.

Inactivation of enzyme activity and chemical modification of native proteins has been reported in polyacrylamide gel electrophoresis even in buffer solutions (35–37). In order to avoid the action of ammonium persulfate, or possible polymerization products of the gelling procedure, or both, which may be detrimental to protein, we previously treated the gels. Initially running the SDS gels with a dye-SDS complex or a low molecular weight protein was attempted in the hope to avoid irreversible inactivation. Both procedures allowed us to detect enzymatic activity after elution and reconstitution. Only slightly lower recovery was found on gels previously treated with the dye crystal violet than with R17 coat protein. Table II summarized the results obtained for the reactivation of four different proteins after SDS gel electrophoresis. Reconstitution from gels results in general in a lower recovery than the reconstitution of enzymes directly from SDS solution, however at least two of the proteins, aspartate transcarbamylase and the σ protein of RNA-polymerase, were recovered in good yield.

DISCUSSION

Six different oligomeric proteins have been successfully reconstituted after dissociation and denaturation by dodecyl sulfate. The procedure involves the removal of SDS by treatment of the protein solution in urea with Dowex 1-X2. Reactivation of the enzymes from the SDS-free urea solution then occurs by dilution into buffer or by dialysis to remove the urea. The recoveries are generally high and quite comparable to those reported in the literature for the renaturation from guanidine-HCl or urea solutions. Refolding of proteins after removal of urea is assumed to be generally accompanied by the re-formation of the native quaternary structure of the enzyme. This was independently shown in our studies by sedimentation velocity analysis of several renatured proteins and kinetic analysis of reconstituted aspartate transcarbamylase. From these results we infer that reconstitution from SDS solution should generally be possible by the two-step procedure outlined above. One potential difficulty in working with SDS can arise in the presence of proteolytic enzymes. As pointed out by Pringle (38) such enzymes may be denatured less rapidly and may degrade the protein of interest. However appropriate precautions have been described to avoid this problem (38, 39). Since this procedure allows one to process small amounts, renaturation of proteins eluted from SDS-polyacrylamide gels seems possible if the gels have been previously treated. Reconstitution from gels resulted in a lower recovery than the reconstitution of enzymes directly from SDS solution. This lower recovery may be accounted for by some residual impurities in the gel matrix, by mechanical losses during the prolonged handling of the samples, or simply by insufficient purity of the different chemicals used in the procedure, all of which may cause a serious problem when small amounts of protein are processed. Current experiments are aimed at optimizing the yields after gel electrophoresis to make the procedure generally more useful. The possibility of oxidation of methionine residues may be avoided by addition of thiodiglycol. Improved yields might also be obtained by using recrystallized chemicals for acrylamide gels and by soaking the gels to remove possible electrophoretically neutral impurities or by-products of the polymerization process.

It is hoped that reconstitution experiments can be used to identify by activity and polypeptide chain molecular weight individual components in complex systems which can be dissociated with SDS. A procedure to characterize by tryptic maps the individual proteins eluted from gels is described.

Acknowledgments—We are grateful to Dr. J. P. Rosenbusch for valuable discussions and to Miss C. Hering, Dr. R. Weil, and Mr. T. Platt for various contributions of different enzymes. We are indebted to Miss M. Simon for excellent technical assistance.

[3] Renaturation of aldolase with Buffer B yielded enzymatically active, high molecular weight aggregates. Upon changing the buffer from Tris-acetate to sodium acetate (Table I), renatured aldolase possessed a sedimentation coefficient identical with that of the native enzyme. Likewise *lac* repressor formed enzymatically active, high molecular weight aggregates upon dilution into Buffer B. No attempts were made, however, to find specific renaturation conditon for *lac* repressor.

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