RAPID AND EFFECTIVE REMOVAL OF SODIUM DODECYL SULFATE FROM PROTEINS

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SUMMARY

Removal of the anionic detergent sodium dodecyl sulfate (SDS) from protein solutions was effected using small columns of Bio-Rad (Dowex) 2-X10. Using [^{35}S]SDS it was shown that more than 99% of the SDS was removed from solutions of reduced ribonuclease, bovine serum albumin and red cell membrane proteins with recovery of 80-95% of the protein. Reduced ribonuclease originally containing 1% SDS and treated as described recovered full enzymatic activity on re-oxidation, indicating that peptide bonds are not destroyed by this procedure. The molecular weight distribution of polypeptide chains of red cell membranes was unaffected by this procedure. About 90% of the red cell membrane phospholipid was also recovered.

The anionic detergent sodium dodceyl sulfate (SDS) has been widely used to disaggregate biological membranes, and to separate their proteins according to molecular weight by gel electrophoresis (e.g., 1-5) and gel filtration (1,6). Further studies on these disaggregated and separated polypeptide chains has been hampered by the difficulty of removing SDS from proteins.

It has been stated in the literature without details that columns of Dowex-2 can be used to remove SDS from disaggregated membranes (7). Removal of SDS was complete as judged by the inability of the column effluent to lyse erythrocytes. In this paper conditions for removal of SDS from solutions of reduced ribonuclease, bovine serum albumin (BSA) and disaggregated human red cell membranes with Bio-Rad 2-X10 has been studied using [^{35}S]SDS. It is

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shown that under appropriate conditions over 99.9% removal of SDS can be effected simply and rapidly with high recovery of protein and without breaking peptide bonds.

Materials and Methods

Bio-Rad AG 2-X10, 200-400 mesh was purchased from Calbiochem in the chloride form, with a capacity of 1.5 meq/ml of resin bed. It was converted to the phosphate form by washing successively with 1 N NaOH, H_2O, 1 M H_3PO_4, H_2O, 0.5 M sodium phosphate buffer pH 7.0 and 0.05 M sodium phosphate buffer pH 7.0 on a sintered glass filter. It was converted to the acetate form by washing successively with 1 N NaOH, H_2O and 0.1 M acetic acid. [^{35}S]SDS was purchased from Amersham-Searle. SDS was recrystallized from water to constant specific activity. BSA, less than 0.01% fatty acid, was purchased from Sigma. Bovine pancreatic ribonuclease and yeast ribonucleic acid were purchased from Worthington. Red cell membranes were prepared from fresh heparinized human blood by the procedure of Dodge, Mitchell and Hanahan (8). The membranes were dialyzed against 5 mM EDTA and 5 mM 2-mercaptoethanol, pH 7.0, and then against water. SDS was added to a final concentration of 1% and the solution heated at 100° for 3 minutes. These conditions are very similar to those previously found to effect complete disaggregation of the membranes into constituent lipids and polypeptide chains (1).

Columns of the resin were prepared in disposable Pasteur pipets. A 3 cm column (volume ca 0.8 ml) was routinely used for a 1 ml sample containing 0.4-5.0 mg of protein in 1% SDS. The sample was eluted with 0.05 M sodium phosphate buffer, pH 7.0 or 0.1 M acetic acid. A total of 3 ml of effluent was collected. Recovery of protein was determined by its OD_{280}. To determine recovery of red cell membrane protein in the absence of urea, it was necessary to add SDS (0.2-0.4% final concentration) to avoid high values of OD_{280} due to light scattering. The columns were poured freshly for each experiment and discarded after a single use.

Ribonuclease was reduced and oxidized as described by Epstein et al.(9) and assayed by the procedure of Anfinsen et al.(10). Gel electrophoresis in SDS was carried out by a method described previously (1).

Organic phosphorus was extracted by method II of Ways and Hanahan (11) and measured by the Bartlett procedure (12). Phospholipids were separated on Schleicher & Schuell silica gel plates, #1500. The plates were first developed with hexane to remove neutral lipids, then developed with chloroform: methanol: acetic acid: water (25:15:4:2). The spots were visualized after drying by reaction with iodine vapor (13).

Results

Table I shows the efficiency of SDS removal from solutions of BSA and red

Table I

Recovery of SDS and Protein From Bio-Rad 2-X10 Columns.

Sample[a]	8 M urea in sample	Column Length	Protein Recovered %	Total cpm of ^{35}S recovered
5 mg BSA[b]	-	1.5	98	312
"	+	1.5	99	27
"	-	3	94	66
"	+	3	97	14
"	-	6	94	30
"	+	6	94	<10
0.6mg BSA	-	3	92	42
"	+	3	95	<10
2 mg. RCM[c]	-	3	73	120
"	+	3	81	640
0.4 mg RCM	-	3	43	66
"	+	3	64	10

[a] All samples were solutions in 1 ml. of 1% [^{35}S] SDS (2.5×10^5 cpm/ml) Samples were eluted with .05 M phosphate buffer, pH 7.0.

[b] BSA =Bovine serum albumin

[c] RCM = red cell membrane protein

cell membrane under several different conditions. Under all the conditions used, over 99% of the SDS was removed by the column. Examination of Table I also reveals the following points: (a) Efficiency of SDS removal from BSA increases as the length of the column is increased; (b) addition of 8M urea increases efficiency of SDS removal from BSA, but has the opposite effect on red cell membranes at the higher concentrations tested; (c) less protein and more SDS is recovered from red cell membrane protein than from BSA; (d) when a lower concentration of red cell membrane proteins is applied to the column, recovery of protein decreases; (e) the amount of SDS recovered decreases as the amount of protein decreases, suggesting that recovered SDS is bound to the protein.

Reduced ribonuclease (2 mg/ml) dissolved in 1% SDS in 0.1 M acetic acid was passed through a column in the acetate form, and the column then washed with 0.1 M acetic acid. No counts could be detected in the effluent of the 1.91×10^5 counts per minute of [^{35}S] applied to the column. Recovery of protein was 80%. This protein regained 100% of ribonuclease activity after air oxidation, as compared with a sample of reduced ribonuclease to which SDS was not added. Samples from which SDS were not removed did not recover any activity after air oxidation.

It is shown in Table II that the amount of SDS which is efficiently removed from a red cell membrane solution is considerably less than would be predicted from the exchange capacity of the resin. The resin capacity of ca 1.2 meq/ml leads to the expectation that a 3 cm column could successfully remove the SDS from as much as 35 ml of 1% SDS (0.0345 M). When a solution of red cell membrane protein in 1% SDS is passed through such a column, however, the efficiency of SDS removal declines continuously, reaching 92% removal after passage of 10 ml (Table II).

Gel electrophoresis of the membrane proteins after passage through the column revealed no detectable differences from the starting material (Fig. 1). About 90% of the phospholipids, as measured by organic phosphorus, was re-

Table II

Recovery of SDS and Red Cell Membrane Protein From A Solution Continuously Applied to A 3 cm. Bio-Rad 2-x10 Column[a]

Fraction #[b]	cpm/ml ^{35}S recovered	Protein recovered %	SDS recovered %
1	44	-	-
2	51	71	0.02
3	74	73	0.03
4	3118	75	1.2
5	10365	77	8

[a] Solution contained 2 mg/ml protein in 1% [^{35}S]SDS (2.65×10^5 cpm/ml)
[b] 2 ml. fractions were collected

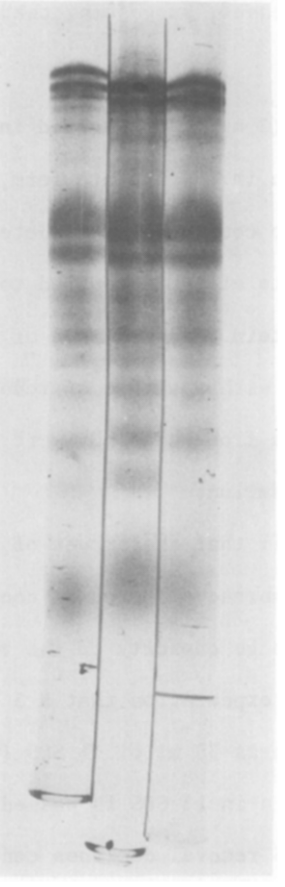

Figure 1. SDS gel electrophoresis of red cell membrane proteins dissolved in 1% SDS before and after passage through Bio-Rad 2-x10 column (3 cm.). From left to right: Before passage through column; after passage through column without urea; after passage through column with 8 M. urea. SDS was added back to column effluents before electrophoresis.

covered in the column effluent. Further, no differences in phospholipid distribution could be detected by thin-layer chromatography after passage through the column. An experiment using columns of Bio-Rad 1-X8 instead of Bio-Rad 2-X10 gave very similar recoveries of protein and SDS, both in the presence and absence of urea.

Discussion

The procedure described in this paper for SDS removal appears to be generally applicable to different kinds of proteins. The efficiency of removal of SDS, however, was better for reduced ribonuclease than for BSA and better for BSA than for red cell membrane proteins (Table I), suggesting that the effectiveness of this technique will vary somewhat for different proteins. Moreover, the fact that the recovery of protein decreases as the protein concentration decreases combined with the fact that the resin does not efficiently bind SDS to its full exchange capacity (Table II) may limit the usefulness of this technique with regard to protein and SDS concentrations which can be successfully treated in this way.

The fact that ribonuclease activity can be fully recovered from reduced ribonuclease treated by the present procedure shows that neither SDS nor the procedure employed for its removal breaks peptide bonds. Also, the degree of removal effected by this procedure was sufficient to permit complete re-oxidation of the reduced protein. However, this procedure might not remove enough SDS to permit renaturation of other proteins. For instance, considering the experiment described in Table I in which 120 counts per minute of [^{35}S]SDS was recovered from a column with 1.46 mg of red cell membrane protein, and assuming a mean molecular weight of 80,000 for the polypeptide chains, there still remains an average of about 1 molecule of SDS still associated with each peptide chain. This amount might be sufficient to prevent proper refolding of one or another of the chains. In any case, the present technique is useful as a rapid and almost quantitative preliminary step.

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