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The Binding of Sodium Dodecyl Sulphate to Various Proteins

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1. The binding of sodium dodecyl sulphate to proteins by equilibrium dialysis was investigated. 2. Most of the proteins studied bound 90–100% of their weight of sodium dodecyl sulphate. 3. The glycoproteins studied bound 70–100% of their weight of sodium dodecyl sulphate, calculated in terms of the polypeptide moiety of the molecule. 4. Proteins not containing S·S groups bound about 140% of their weight of sodium dodecyl sulphate. 5. Reduction of four proteins containing S·S groups caused a rise in sodium dodecyl sulphate binding to 140% of the weight of protein. 6. The apparent micellar molecular weights of the protein–sodium dodecyl sulphate complexes were measured by the dye-solubilization method; they were all found to have approximately the same micellar molecular weight (34000–41000) irrespective of the molecular weight of the protein to which they were attached.

During experiments on the reduction of thyroglobulin in SDS† (R. Pitt-Rivers & F. S. Ambesi Impiombato, unpublished work) it became necessary to determine the amount of detergent bound to the reduced and unreduced protein. We found that, if the protein was dialysed against a buffer containing SDS until equilibrium had been reached, the weight of SDS bound almost equalled the weight of protein. Such binding considerably exceeded the binding of SDS to proteins previously described, e.g. serum albumin (Pallansch & Briggs, 1954; Hunter & McDuffie, 1959; Reynolds, Herbert, Polet & Steinhardt, 1967). It was therefore decided to investigate the binding of SDS to other proteins under the conditions used for our experiments on thyroglobulin.

MATERIALS AND METHODS

The following were obtained from commercial sources. SDS (specially pure), rosaniline hydrochloride, β-lactoglobulin and iodoacetamide were from British Drug Houses Ltd., Poole, Dorset; the iodoacetamide was recrystallized from water. Sudan Yellow, *o*-tolylazo-β-naphthol (Orange OT in the American literature), was from George T. Gurr Ltd., London, N.W. 9; this was purified by the method of Williams, Phillips & Mysels (1955). Dithiothreitol was from Calbiochem Ltd., London, W. 1. Lysozyme was from Sigma (London) Chemical Co. Ltd., London, S.W. 6. Ovalbumin (three times crystallized) was from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. Catalase (crystalline suspension) was from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. BSA was from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex. Ribonuclease was from Worthington Biochemical Corp., Freehold, N.J., U.S.A.

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Pig thyroglobulin was prepared by the method of Brownstone (1968). Rat methaemoglobin was prepared by washing erythrocytes with 0·9% NaCl until the solvent was free from protein, and lysing them with distilled water; after removal of the erythrocyte 'ghosts' by centrifugation, the haemoglobin was converted into methaemoglobin with ferricyanide.

Equilibrium dialysis of proteins. Crystalline or freeze-dried proteins were weighed and dissolved in 66mM-sodium phosphate buffer, pH 7·2, at a concentration of 1·0%; solutions of proteins were brought to 1·0% concentration by using their molar coefficients of extinction to calculate concentration from data obtained from Dr G. H. Beaven. Samples (about 5ml.) of the protein solutions were placed in Visking 8/32 sacs; the tubing was pretreated by soaking it for at least 48hr. in 1·5% (w/v) SDS solution, and thoroughly washed with hot tap water and distilled water (C. G. Knight, personal communication). Dialysis was carried out against at least 500vol. of 0·1% (w/v) SDS in 66mM-sodium phosphate buffer, pH 7·2, containing 0·02% NaN_3, with continuous stirring at room temperature. In experiments in which the SDS solution contained added NaCl, the SDS concentration was lowered to below the critical micelle concentration for the particular salt solution

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† Abbreviations: SDS, sodium dodecyl sulphate; BSA, bovine serum albumin; BγG, bovine γ-globulin; T&H glycoprotein, Tamm and Horsfall urinary glycoprotein; glycoprotein 376, blood-group substance cyst 376 glycoprotein.

(see Williams *et al.* 1955). For proteins with mol.wt. lower than 25000 the Visking 8/32 tubing was acetylated by soaking it in 10% (v/v) acetic anhydride in pyridine for 16hr. at room temperature. At approximately daily intervals, samples of the proteins were removed and analysed for SDS and protein. Equilibrium was considered to have been reached when the ratio SDS/protein did not change.

In one case (glycoprotein 376) dialysis of a 0·4% solution was carried out in 50 mM-sodium acetate buffer, pH 4·6, to conform with the previous studies by Creeth & Knight (1967).

Because of the insolubility of certain proteins in concentrations of SDS between 0·1 and 0·5% (see Putnam, 1948; Putnam & Neurath, 1944), the following were dissolved in an initial concentration of 0·5% (w/v) SDS: β-lactoglobulin, apomyoglobin, lysozyme and T&H glycoprotein.

Reversal of SDS binding. A number of the protein–SDS complexes were dialysed against repeated changes of SDS-free buffer until SDS could no longer be detected in the diffusate or until precipitation of the protein occurred. The proteins that remained in solution were analysed for bound SDS.

Determination of SDS. The method of Karush & Sonenberg (1950) as modified by Pallansch & Briggs (1954) was later adapted by Hunter & McDuffie (1959) for the determination of SDS attached to proteins. For our experiments, it was found necessary to scale down this method as follows: 0·1–0·2ml. of the 1% protein solution was treated with 1 drop of 0·5M-acetic acid and shaken in a centrifuge tube with 5ml. of acetone. The precipitated protein was removed by centrifuging and the acetone extract of SDS transferred to a round-bottomed flask. The protein precipitate was resuspended in 0·2ml. of 1M-sodium acetate buffer, pH 4·0, containing 1M-NaCl, and re-extracted with 5ml. of acetone. The pooled acetone extracts were evaporated to dryness and the SDS was extracted from the flask with several washes of 0·66mM-sodium phosphate buffer, pH 6·1, and made up to 25ml.; 0·2ml. of this solution was diluted with 3·8ml. of the phosphate buffer, pH 6·1. Then 1ml. of 0·4mM-rosaniline hydrochloride solution [previously exhaustively extracted with chloroform–ethyl acetate (1:1, v/v)] was added, and the SDS rosaniline salt was extracted into 5ml. of chloroform–ethyl acetate (1:1, v/v) by shaking 50 times. After centrifugation, the SDS in the organic phase was measured in a Unicam SP.500 spectrophotometer from its extinction at 545mμ; the value was obtained from a standard curve prepared by the method of Pallansch & Briggs (1954). For calculation of the bound SDS, it was assumed that the free SDS inside the dialysis sac was equal to that outside at equilibrium.

Reduction of ovalbumin, β-lactoglobulin, ribonuclease and ovomucoid. The proteins were dissolved at 1% concentration in 50mM-glycine–16·6mM-tris buffer, pH 8·7, containing 3mg. of SDS/ml., and reduced for 4hr. with dithiothreitol (8mg./ml.); iodoacetamide was then added in 30% excess over the thiol groups present, and allowed to react for 1½hr. Excess of carboxamidodithiothreitol was removed by dialysis against 66mM-sodium phosphate buffer, pH 7·2, containing 0·1% SDS and 0·02% NaN_3. The buffer was changed several times before protein and SDS concentrations were determined.

Succinylation. Succinylation of BSA was performed by the method of Habeeb, Cassidy & Singer (1958).

Measurement of micelle formation by protein–SDS complexes. The solubilization of water-insoluble dyes by detergent micelles (Hartley, 1936; McBain, 1942) has been used to determine their critical micelle concentration (Williams *et al.* 1955) and micellar molecular weight (Schott, 1966). Although there are theoretical objections to the latter determination, which are discussed below, it was thought worth while to study the solubilization of Sudan Yellow by the protein–SDS micelles obtained by equilibrium dialysis, to find out whether any estimate of their size were possible. Protein–detergent solution (1ml.) was diluted with 4ml. of the appropriate SDS–buffer solution and was shaken for 18hr. at room temperature with an excess of solid Sudan Yellow. Undissolved dye was separated by centrifuging and the extinction of the solution at 498mμ was determined in a Unicam SP.500 spectrophotometer, with the SDS–buffer saturated with the dye in the reference cell. Solutions of the proteins alone did not solubilize the dye, with the exception of BSA, which gave a slight positive colour, presumably due to contaminating serum lipids. The critical micelle concentration (C.M.C.) of SDS was determined for all the buffer–salt solutions used by the method of Williams *et al.* (1955). The micellar molecular weight (M.M.W.) of the micelles was calculated by Schott's (1966) method from the formula:

$$\text{M.M.W.} = (c - \text{C.M.C.})l\epsilon/E$$

where c = concentration of SDS (g./l.) obtained from the standard curves, l = light path of cell (cm.), E = extinction at 498mμ and ϵ = molar extinction coefficient of Sudan Yellow in SDS, found by Schott (1966) to be $1{\cdot}994 \times 10^4$l./mole.

RESULTS

The binding of SDS to protein and the time taken to reach equilibrium are shown in Table 1. The proteins are divided into two groups: those that contain S·S groups and those that do not. Data on molecular weight and, where pertinent, carbohydrate content are also given. For the glycoproteins, the bound SDS is calculated as a function of the polypeptide backbone of the molecule only. Both the amount of SDS bound and the rate of binding varied between the two groups; the proteins without S·S groups bound approximately 50% more SDS than did the proteins with S·S groups, and in about half the time. The rapid binding of SDS by lysozyme and β-lactoglobulin was due to the fact that the initial SDS concentration was already 0·5%; the fairly rapid binding of SDS by glycoprotein 376 may be attributed to the fact that the protein moiety of this molecule possesses no secondary structure (Creeth & Knight, 1967). The rather slow binding of SDS by catalase may be due to slow unfolding of the large molecule. Polylysylglutamic acid at pH 7·2 bound only 0·4mg. of SDS/mg. of polypeptide.

The possibility that the smaller binding of SDS by proteins with S·S bonds might be due to restriction of their unfolding was investigated by reduction and alkylation of four of them. The results are

Table 1. *Binding of SDS to proteins*

The mol.wt., half-cystine and carbohydrate data are from the literature, as indicated. For the glycopeptides, the SDS/protein ratios are calculated as a function of the polypeptide moiety only. For experimental details and abbreviations, see the text.

Protein	Mol.wt.	Half-cystine (residues/molecule)	Carbohydrate (%)	SDS/protein ratio (mg./mg.)	Time to reach equilibrium (days)
With S·S groups					
T&H glycoprotein	6×10^6*	4/28000*	30*	0·66	10
Glycoprotein 376	5×10^6†	—	84†	1·0	5
Thyroglobulin	660000‡	200‡	10‡	1·1	8
BγG	160000¶	4¶	3¶	0·95	10
BSA	66000¶	34¶	0¶	0·93	10
Ovalbumin	45000¶	2¶	3¶	0·90	8
Ovomucoid	30000§	16§	25§	0·73	10
β-Lactoglobulin	18000‖	2‖	0‖	0·90	4
Lysozyme	14000¶	8¶	0¶	1·0	4
Ribonuclease	13700¶	4¶	0¶	0·92	9
Without S·S groups					
Catalase	250000¶			1·38	7
Methaemoglobin	64000¶			1·38	2
Metmyoglobin	17800¶			1·40	4
Apomyoglobin	17200¶			1·34	4

* Maxfield (1966); † Pusztai & Morgan (1963); ‡ Edelhoch & Rall (1964); § Melamed (1966); ‖ Bull (1946); Timasheff (1964); ¶ Neurath (1964).

Table 2. *Effect of reduction and alkylation on the binding of SDS to proteins with* S·S *groups*

For experimental details, see the text.

Protein	SDS/protein ratio (mg./mg.) Before reduction	After reduction
Ovomucoid	0·73	1·42
Ovalbumin	0·90	1·40
β-Lactoglobulin	0·90	1·39
Ribonuclease	0·92	1·39

given in Table 2 and show that, after the cleavage of S·S bonds, the uptake of SDS reached the same maximum as that obtained with the proteins without S·S bonds.

Reversal of SDS binding. After partial removal of SDS, haemoglobin, myoglobin, apomyoglobin, BγG and lysozyme were precipitated from solution and were not further investigated. After exhaustive dialysis against SDS-free buffer, thyroglobulin, ovalbumin and ribonuclease remained in solution although no SDS could be detected in the contents of the dialysis sac. The limit of detection of SDS by the method used is between 1 and 2 μg./ml. We therefore conclude that virtually all the SDS had been removed from the proteins. These findings are in contrast with those of Putnam & Neurath (1944), who were unable to remove detergent from protein–SDS complexes by prolonged dialysis, although they were able to do so by addition of barium chloride, which forms an insoluble barium salt of SDS. Even the latter method is not always successful; McMeekin, Polis, Della Monica & Custer (1949) recorded the formation of a stable crystalline complex of two molecules of SDS/molecule of β-lactoglobulin from which they were unable to dissociate the detergent by dialysis or by treatment with barium hydroxide.

Effect of added salt on SDS binding. Binding of SDS to thyroglobulin, BγG and methaemoglobin was determined in 66mM-sodium phosphate buffer, pH 7·2 to which was added 50mM- or 0·154M-sodium chloride. The results are shown in Table 3. The binding of SDS to thyroglobulin and BγG was considerably accelerated in the presence of 50mM-sodium chloride; however, 0·154M-sodium chloride markedly slowed the binding process and decreased the amount of detergent bound to BγG and haemoglobin; this might have been caused by charge effects that could inhibit unfolding of the protein molecule. In this connexion, it is noteworthy that at pH 7·2 the highly charged polylysylglutamic acid bound only 0·4mg. of SDS/mg. of polypeptide, and that succinylation of BSA decreased the binding of SDS at pH 7·2 from 0·93 to 0·61mg./mg. of protein.

Table 3. *Effect of added salt on SDS binding*

For experimental details and abbreviations, see the text.

Concn. of NaCl	0		50mM		0·154M	
Protein	SDS/protein ratio (mg./mg.)	Time for maximum binding (days)	SDS/protein ratio (mg./mg.)	Time for maximum binding (days)	SDS/protein ratio (mg./mg.)	Time for maximum binding (days)
Thyroglobulin	1·0	8	1·0	3	Protein precipitated	
BγG	0·95	12	0·9	6	0·38	20
Methaemoglobin	1·38	3	—	—	0·46	7

Table 4. *Apparent micellar molecular weight of SDS in complexes with proteins, measured by dye solubilization.*

For experimental details and abbreviations, see the text.

Protein	Micellar molecular weight per dye molecule
T&H glycoprotein	41000
Thyroglobulin	38000
BγG	34000*
Ovalbumin	38000
Ovomucoid	35000
Apomyoglobin	36000
Lysozyme	38000
Polylysylglutamic acid	39000

* The buffer used in these experiments (66mM-sodium phosphate buffer, pH7·2) was supplemented with 0·154M-NaCl.

Relative micellar molecular weights of SDS–protein complexes. The large amount of SDS bound to protein (Table 1) suggested that it was present as hemimicelles containing a core of polypeptide chain; determination of the micellar molecular weights by the method of Schott (1966) was carried out. Calculation of micellar molecular weight by this method is based on the assumption that the micelle of SDS solubilizes a constant number of dye molecules; Schott (1966) assumed this number to be one (for SDS, but not for other detergents) since the micellar molecular weight found by him (about 37000) agreed with values found by different methods reported in the literature (see below). The micellar molecular weight of SDS and of other detergents is influenced by the nature and concentration of the supporting electrolyte. This has been shown by light-scattering, equilibrium-ultracentrifugation and other methods (Phillips & Mysels, 1955; Anacker, Rush & Johnson, 1964). With SDS, the micellar molecular weight rises from about 22000 in salt-free medium or 0·1M-sodium chloride to about 36000 in 0·4M-sodium chloride (see Fig. 8 in Anacker *et al.* 1964). Anacker (1968) pointed to the anomalous finding in Schott's (1966) experiments that the micellar molecular weight of SDS is significantly lower in high-salt than in low-salt medium, and criticized the comparison of micellar molecular weights that are measured in different electrolyte concentrations. For these and other reasons (Mysels, 1967), the assumption that one SDS micelle will dissolve one dye molecule is probably not justifiable; we have therefore expressed the apparent micellar molecular weights of the protein–SDS complexes as a function of the dye solubilized. The results are shown in Table 4. The apparent micellar molecular weights of these complexes in 66mM-sodium phosphate buffer, pH7·2, were similar to each other and to the micellar molecular weight of SDS alone, in 0·4M-sodium chloride. Addition of 0·15M-sodium chloride, in the case of BγG, did not significantly alter the micellar molecular weight.

DISCUSSION

Several studies on the binding of detergents to protein have shown that the nature of the binding varies according to the amount of detergent bound. Putnam & Neurath (1945) found that two electrophoretically stable complexes could be formed between horse serum albumin and SDS, containing 55 and 110 SDS molecules/molecule of protein. A third complex containing 220 SDS molecules/molecule of albumin was unstable on electrophoresis, and on cooling to 1° broke down to form insoluble SDS and the complex containing 55 SDS molecules/molecule. Pallansch & Briggs (1954), in short-term dialyses, obtained an SDS–BSA complex not exceeding 40 molecules of detergent/molecule of BSA. Hunter & McDuffie (1959) found that SDS bound to human serum albumin in the molar ratio 63–67:1, and that reduction of the S·S group of the protein increased the binding to 92–94:1. Reynolds *et al.* (1967) have shown that native BSA possesses ten primary binding sites for SDS and that the

protein is only grossly disorganized when the SDS/protein molar ratio is increased above 10.

In our experiments, the molar ratio of SDS to BSA at equilibrium was 200:1, which would give a molecular weight for the complex of 125000. Harrap & Schulman (1953) studied by light-scattering the molecular weights of different BSA–SDS complexes in water and dilute (50mM) sodium chloride solution, and found that the molecular weight of the complex containing 200 SDS molecules was 131000; these authors quote Cockbain's (personal communication to Harrap & Schulman, 1953) finding, obtained by the action of SDS on the interfacial tension between a solution of BSA and benzene, that the molar ratio of SDS to protein was 220; our results with BSA are therefore in good agreement with the maximal binding of SDS to serum albumin previously reported.

Our results on the binding of SDS to glycoprotein 376 are not in agreement with a previous finding: if we calculate the molar ratio of SDS bound in 50mM-sodium acetate buffer, pH4·6, to this protein (including carbohydrate), it is about 50:1; Creeth & Knight (1967) from ultracentrifuge data obtained the same value only in buffer of low ionic strength (10mM-sodium acetate, pH4·6), but showed that it was increased to 120:1 when the ionic strength of the buffer was raised to 50mM–0·4M.

The results of our experiments on the apparent micellar molecular weight of the protein–SDS complexes are difficult to explain; the fact that the micellar molecular weights are all very similar, irrespective of the molecular weight of the protein to which they are attached, suggests that the detergent grows round the binding site on the protein backbone to form a micelle whose size is limited by the chemical and physical properties of the detergent and by the ionic environment. With proteins of molecular weight exceeding 20000, one could envisage the formation of beads of micelles, strung along the polypeptide backbone. These experiments provide evidence that there is a structural similarity between SDS micelles and the SDS adsorbed on protein, since the two possess similar solubilizing power for the dye Sudan Yellow.

Certain proteins, such as thyroglobulin (Edelhoch & Lippoldt, 1960), are dissociated by SDS into sub-units; others, such as human serum albumin (Hunter & McDuffie, 1959) and β-lactoglobulin (P. A. Charlwood, personal communication), form SDS complexes which appear as symmetrical peaks in the ultracentrifuge.

The fact that polylysylglutamic acid bound only 0·4mg. of SDS/mg. of polypeptide although the apparent micellar molecular weight was the same as that of other complexes suggests that certain primary binding sites on the polypeptide backbone were blocked by the charge effect, but that the subsequent formation of SDS micelles was not affected.

From our results, it appears that the binding of SDS to protein is relatively non-specific in a wide variety of proteins; it appears to be a function of the polypeptide moiety of the proteins, as seen from the results with the glycoproteins, and is not influenced by the presence of large amounts of carbohydrate. Maximum binding of detergent depends on its ability to unfold the protein, and is restricted by the presence of S·S groups; this was indicated by the work of Hunter & McDuffie (1959), although neither in whole nor in reduced human serum albumin was binding of SDS maximal. As shown above, the binding by SDS to protein is influenced by salt concentration and the charge on the protein (polylysylglutamic acid and succinylated BSA).

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