The Interaction of a Cationic Detergent with Bovine Serum Albumin and Other Proteins

(Received for publication, November 7, 1973)

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SUMMARY

The binding of tetradecyltrimethylammonium chloride to bovine serum albumin and several other globular proteins was measured, and the resulting conformational changes were studied by optical and hydrodynamic techniques. Native serum albumin was found to have four discrete binding sites for the detergent, to which binding occurs without conformational change, and with a much lower binding constant than for anionic detergents with alkyl chains of similar length. At higher detergent concentrations the cationic detergent resembles sodium dodecyl sulfate, binding cooperatively to serum albumin and to all other proteins studied, with accompanying gross denaturation to form extended rod-like complexes. However, this cooperative process occurs for the cationic detergent at a 10-fold higher concentration than is required for sodium dodecyl sulfate, with the result that completion of the transition was prevented (under the conditions used) by the onset of micelle formation. A possible explanation is offered for this difference, which probably applies to all cationic detergents. The results suggest that cationic detergents are not suitable as substitutes for sodium dodecyl sulfate in procedures in which the denaturing action of the detergent is an essential feature, as, for example, in the determination of molecular weight by gel chromatography or gel electrophoresis.

Previous studies using hydrocarbons, anionic and neutral detergents, and other amphiphilic substances (1–7) have shown that these substances can combine with proteins in at least three quite distinct modes of interaction:

1, Association with specific binding sites of native proteins. Two common water-soluble proteins, serum albumin and $\beta$-lactoglobulin, possess such sites, but many other proteins, e.g. ovalbumin and hemoglobin, do not. The binding sites of serum albumin and $\beta$-lactoglobulin differ in their specificity: the single site of $\beta$-lactoglobulin is primarily hydrophobic in character and can combine with pure hydrocarbons as well as with am-

phiphiles, whereas the 10 (or more) binding sites of serum albumin involve electrostatic as well as hydrophobic affinity and show strong specificity for anionic amphiphiles.

2, Cooperative association between protein and a large number of detergent molecules without major conformational change. This type of reaction has been observed as occurring between serum albumin and alkyl sulfates and sulfonates with relatively short hydrocarbon chains. Scattered experiments carried out in this laboratory suggest that this type of reaction is quite common, but detailed studies have not been made.

3, Cooperative association with gross denaturation of the protein. This reaction, studied in greatest detail with sodium dodecyl sulfate ($C_{12}OSO_3^{-}$), occurs with all proteins. The native structure is destroyed and replaced by an extended rod-like conformation with a moderately high content of $\alpha$ helix, in which most of the hydrophobic residues are presumably exposed for association with the detergent. Reduction of disulfide bonds is required to optimize availability of hydrophobic residues and to obtain maximal binding.

In all of these reactions, the thermodynamic activity of the detergent is determined by the concentration of monomeric molecules, and detergent micelles do not contribute. Micelles and protein molecules can of course associate with each other, by polar bonds between the protein and the micelle surface (8), but this type of interaction cannot occur with ionic detergents that bind copiously to protein below the critical micelle concentration (cmc) because coexisting micelles and protein complexes then have large charges of like sign.

The two cooperative modes of association described above require a considerably higher monomeric detergent concentration than is required for association with native binding sites, where they exist. The thermodynamics of micelle formation thus becomes an important factor: it is reasonable to believe that all detergents are capable of cooperative association with proteins, but detergents with a relatively low cmc may not be able to reach a sufficiently high monomer concentration to do so (7). Since ionic detergents have a considerably higher cmc than nonionic detergents with hydrocarbon chains of the same length (6), as a result of the stronger repulsion between

1 The abbreviations used are: cmc, critical micelle concentration; $C_{1}NMe_3^{+}$, tetradecyltrimethylammonium chloride; $C_{12}O_{2}S_{2}O_{3}^{-}$, sodium dodecyl sulfate; $C_{n}NMe_3^{+}$, alkyltrimethylammonium chloride or bromide with an alkyl chain of $n$ carbon atoms. The omission of the counterion in the abbreviations for detergents is not intended to imply that the counterion is entirely without influence on the results.

* This work was supported by Research Grants GB-14644 from the National Science Foundation and AM-04576 from the United States Public Health Service.

† Recipient of a Research Career Award, National Institutes of Health, United States Public Health Service.
ionic head groups, the cooperative modes of association are more likely to be observable for ionic than for nonionic detergents.

Only a few comparable studies of cationic detergents have been reported. A preliminary study of the association between C12NMe3+ and serum albumin (9) indicates the occurrence of cooperative binding with denaturation, and electrophoretic studies (10, 11) suggest that C10NMe3+ may combine with diverse proteins, and that the electrophoretic behavior of the resulting complex resembles that of the saturated complex between proteins and sodium dodecyl sulfate. On the other hand, rhodopsin extracted from retinal rods with C12NhIe3f or ClsN-Ve3+ appears to retain its native structure if the temperature is kept low (12, 13). There is spectroscopic evidence for cooperative association between glicolipid and C10NMe3+ and C12NMe3+, with accompanying conformational change (14).

We report in this paper a somewhat more detailed study of a cationic detergent of the same type as was used in the experiments cited above, namely C10NMe3+. We chose a tetradecyl alkyl chain as the shortest chain likely to ensure that cooperative binding with denaturation, and electrophoretic behavior of the resulting complex resembles that of the saturated complex between proteins and sodium dodecyl sulfate. In the interaction between alkyl sulfates and unreduced serum albumin the transition between cooperative binding with and without extensive unfolding occurs between C10 and C12 alkyl chains (2), but when alkyl sulfonates are used a C10 chain is not sufficient and there are grounds for believing that 1 extra carbon atom is needed (6).

**EXPERIMENTAL PROCEDURE**

**Materials**—n-Tetradecyltrimethylammonium chloride (C14NMe3+) was purchased from Lachat Chemicals, Inc., Chicago Heights, Ill. It was recrystallized from methanol solution by addition of ether, and stored in a vacuum desiccator. The recrystallized material melted at 237.5 to 239.5° (with decomposition) and contained no detectable impurity by thin layer chromatography. 14C-Labeled C10NMe3+ was obtained from Amersham-Searle Corp., Arlington Heights, Ill. Its radiochemical purity on the basis of thin layer chromatography was 97%, and it was used without further purification. Detergent used for binding studies usually contained the radioactive compound at a level of 1 part to more than 106 parts of the unlabeled reagent.

Bovine serum albumin, 4 times crystallized, and ovalbumin, 5 times crystallized, were purchased from Nutritional Biochemical Corp. Human immunoglobulin and conalbumin, 6 times crystallized, were purchased from Schwarz-Mann. The latter was found not to be iron-free (as specified) and was treated to remove all traces of metal. Bovine carbonic anhydrase B, purified by chromatography on DEAE-Sephadex, was a gift from R. W. Henkens, Duke University. All proteins, as finally used, were found to be free from contamination of polyepitides by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Reduced and carboxymethylated proteins were prepared by standard procedures in 6 M guanidine hydrochloride, pH 8. They were dialyzed exhaustively against distilled water, which usually led to precipitation of the protein, and were redissolved in detergent at the desired concentration. Protein concentrations were determined spectrophotometrically, using absorptivities based on accurate dry weight analyses.

**Critical Micelle Concentration**—The critical micelle concentration was determined as a function of buffer concentration in Tris chloride, pH 8.1, at 24°-25°. The results are shown in Fig. 1 and they agree reasonably well with similar data by Kushner et al. (17), who used NaCl to vary the ionic strength. A slightly higher cmc was obtained in one experiment using phosphate buffer, pH 6.0. Such variation in the cmc depending on the counterion employed is commonly observed, e.g. cmc values for C10NMe3+ are slightly lower when bromide replaces chloride as counterion (19).

On the basis of light-scattering and viscosity measurements of Kushner et al. (17) the micelles formed under the conditions of our binding studies are globular, with an average aggregation number of about 60 to 80. Micelle formation is thus a highly cooperative process, though of course not as sharp as a true phase separation. The concentration of monomeric detergent continues to rise above the cmc, though not by as much as in the

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**RESULTS**

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Critical micelle concentration of $\text{C}_{14}\text{NMe}_3^+$ (chloride) at 23-25°C. ○, this work, Tris chloride buffer, pH 8.1; △, this work, phosphate buffer, pH 5.6; ▽, Cella et al. (18), no added salt; ■, Kusher et al. (17), NaCl. All concentrations are in units of moles per liter.

**FIG. 2** (right). Relation between monomer concentration and the cmc. The curve is based on an assumed equilibrium between monomeric detergent and micelles containing 80 detergent molecules per micelle. The cmc is taken to correspond to the total detergent concentration at which 2% of the detergent is in micellar form.

**TABLE I**

| Association constants for native bovine serum albumin at 23-25°C |
|---------------------------------------------------------------|
| Number of sites | $K$ (liter/mole) |
| 4 | $1.5 \times 10^4$ |
| 4 | $8 \times 10^4$ |
| 8-10 | $1.1 \times 10^6$ |
| 10-11 | $1.5 \times 10^6$ |

a Refs. 2 and 21.
b According to Halfman and Nishida (22) there are 12 sites, which can be subdivided into four sites with $K = 10^7$ and eight sites with $K = 10^8$ liters per mole.

detergent concentration. Only the cooperative interaction is observed for ovalbumin. A Scatchard plot (20) of the results for serum albumin shows that the first phase of binding can within experimental error be accounted for in terms of four identical and noninteracting sites. The binding constant derived from this treatment is shown in Table I, and the solid line in Fig. 3 is a theoretical binding curve based on it. Quite similar results were obtained in 0.03 M Tris chloride buffer, pH 8.1, yielding the same number of sites but a higher association constant. The results are compared in Table I with previous results for the binding of $\text{C}_{14}\text{OSO}_3^-$. It is evident that the sites for binding of the cationic detergent are smaller in number and weaker in affinity. Cationic detergent binding also seems to have a more marked pH dependence.

The cooperative mode of binding is examined in more detail in Fig. 4. This figure shows an extension of the binding data for serum albumin and ovalbumin to higher detergent concentrations, with binding expressed on a weight instead of a molar basis. Data are also given for the same two proteins after reduction and carboxymethylation, and single points are included for reduced and carboxymethylated conalbumin and human immunoglobulin G.

The figure includes data obtained above the cmc of the detergent, which, as explained under "Experimental Procedure," are subject to considerable uncertainty. The points shown in parentheses, indicating an apparent decrease in binding with in-
increasing detergent concentration, are thermodynamically inadmissible and have to be artifacts arising from a failure to attain equilibrium. The underlying cause is likely to be a clogging of the dialysis membrane pores by detergent micelles; we have not attempted to demonstrate this by repeated reequilibration with fresh membranes. Most of the results between the cmc and log \( C = -2 \) represent multiple experiments in which convergence to a single equilibrium state from several initial concentrations was demonstrated. Some of the solutions were heated to 100° for 10 min before equilibration to accelerate the conformational transition that accompanies cooperative binding. These solutions gave the same result, within experimental error, as comparable unheated solutions. Below the cmc, where the time for equilibration across the dialysis membrane is reasonably short, a true reversibility test was carried out, i.e. a solution that had come to equilibrium at close to the maximal binding level was re-equilibrated by dialysis against lower detergent concentration. Final equilibrium data agreed within experimental error with points obtained initially at the same free detergent concentration.

As will be shown below, the cooperative mode of binding is accompanied by a drastic conformational change, thereby resembling the similar process in \( C_4\text{OSO}_4^- \) (2). The cationic and anionic detergents are also similar in other respects, i.e. the detergent concentration at which cooperative binding sets in is about the same for a variety of proteins, and the conformational restrictions imposed by disulfide bonds lead to a lower level of binding than is observed when disulfide bonds are broken (4, 15). However, whereas two discrete levels of saturation of reduced proteins have been observed for sodium dodecyl sulfate (4), only a single cooperative stage is seen here.

The most important difference between the two detergents lies in the fact that the cooperative mode of binding for the cationic detergent occurs very close to its cmc, whereas the corresponding process for dodecyl sulfate, at a similar low ionic strength, occurs well below the cmc. (The low ionic strength used for these experiments was designed, on the basis of the results for dodecyl sulfate, to give maximal advantage to association with the protein as compared to self-association to form micelles.) A consequence of this is that the apparent leveling off of the binding isotherms at high detergent concentration probably does not represent saturation of the protein, but is more likely to be the result of the abrupt leveling off of the thermodynamic activity of the unbound detergent, which, neglecting thermodynamic nonideality, may be equated with the concentration of detergent monomer. This is illustrated in Fig. 5, where the results for reduced and carboxymethylated ovalbumin and serum albumin have been replotted as a function of the monomeric detergent concentration in equilibrium with the protein, estimated from Fig. 2, in place of the total unbound detergent concentration used in Fig. 4. No leveling off of the isotherm is seen.

Additional evidence that the onset of micelle formation prevents reaching saturation in the cooperative binding is obtained from preliminary measurements in Tris chloride buffer at pH 8.1. The cooperative binding occurs at somewhat lower detergent concentration at this pH, reflecting a pH dependence similar to that observed for \( C_4\text{OSO}_4^- \) (Fig. 10 in Ref. 21), except that the direction of change is reversed. The maximal binding of \( C_4\text{NMe}_3^+ \) attained in these studies was higher than at the lower pH, e.g. for reduced and carboxymethylated proteins, maximal values of 0.9 to 0.95 g of detergent per g of protein were found, whereas maximal values in Fig. 4 are about 0.8 g per g. The simplest explanation is that the difference results from displacement of the cooperative transition relative to the cmc, and that neither figure represents saturation.

The inability to determine the saturation level of binding obviously limits the usefulness of these data, and it is for this reason that we have not tried to improve the precision of the data of Fig. 4 at the highest detergent concentrations.

Optical Measurements—Fig. 6 shows optical rotatory dispersion measurements for bovine serum albumin at several levels of binding of \( C_4\text{NMe}_3^+ \), and a comparable measurement in concentrated guanidine hydrochloride. The results are similar to those obtained previously for other detergents (7). Noncooperative binding to discrete sites of the native protein produces no detectable change in conformation, but binding in the cooperative mode is accompanied by denaturation. The denatured state is not a random coil (as in guanidine hydrochloride), but a highly structured state. The trough at 233 nm is often considered to reflect the presence of an \( \alpha \) helical conformation, and by this criterion the denatured state of bovine serum albumin with disulfide bonds intact has a lesser helix content than the native state. Circular dichroism measurements shown in Fig. 7
lead to the same conclusion. The double trough between 200 and 250 nm is characteristic of \( \alpha \) helical polypeptides (23), but its lack of symmetry indicates that other structural features contribute to the spectrum.

Fig. 7 includes results for reduced and carboxymethylated bovine serum albumin and comparable measurements in \( \text{C}_{12}\text{SO}_{4}\text{O}^- \) solutions. Since the disulfide bond itself makes no significant contribution to the dichroism between 200 and 250 nm (24), the results indicate that the detergent-denatured protein undergoes further structural change when disulfide bonds are reduced. The figure also shows that the over-all conformational changes associated with binding of \( \text{C}_{12}\text{NMe}_3^+ \) are very similar to those that occur in the cooperative binding of \( \text{C}_{12}\text{SO}_3^- \).

Similar studies have been made with several other proteins. For ovalbumin, which has a low apparent helix content in the native state, the double trough between 200 and 250 nm is deeper in the denatured state, both in \( \text{C}_{12}\text{NMe}_3^+ \) and \( \text{C}_{12}\text{SO}_3^- \), in contrast to what is observed for bovine serum albumin. This result is consistent with the increase in apparent helix content of ovalbumin in \( \text{C}_{12}\text{NMe}_3^+ \) and \( \text{C}_{12}\text{SO}_3^- \) reported on the basis of optical rotatory dispersion measurements by Meyer and Kaufmann (25). We have found that carbonic anhydrase, which also has a low helix content in the native state, behaves like ovalbumin. Similar results in \( \text{C}_{12}\text{SO}_3^- \) have been reported for many proteins (5, 26, 27). It seems to be a general result that the optical parameters associated with \( \alpha \) helical polypeptide chains are at an intermediate level in detergent-denatured proteins, being increased upon denaturation when the native protein has a low apparent helix content, and diminished when the native protein is highly helical.

The inset of Fig. 7 shows near ultraviolet circular dichroism spectra of bovine serum albumin. The prominent negative dichroic band near 260 nm (Curves 1, 3, and 5) is at too low a wavelength to be ascribed to the aromatic absorption bands of tyrosine and tryptophan, and it is likely that the conformational asymmetry of the disulfide bonds makes a major contribution to it (28). (The very small waviness superimposed on the trough between 250 and 270 nm is almost within the experimental error of measurement, but would be in the right position to be attributed to phenylalanine residues.) The observed magnitude of the trough is larger than that measured for the model cystine derivative, \( \text{N,N'}\text{-diacetyl-L-cystine bismethylamide} \) (24). The maximal mean residue ellipticity for bovine serum albumin corresponds to a molar ellipticity of about \(-4000\) deg cm\(^2\) per dmole per disulfide bond, whereas the molar ellipticity of the model compound is about \(-1000\) deg cm\(^2\) per mole at room temperature. Fig. 7 shows that the dichroic band for bovine serum albumin is not affected significantly by denaturation by detergent, but that it is eliminated by reduction and carboxymethylation, in the absence or presence of detergent. It is noteworthy that guanidine hydrochloride denaturation, without rupture of...
DISCUSSION

Interaction with Native Proteins—The binding sites of native serum albumin for large organic ligands are known to be non-specific. They are generally visualized as hydrophobic areas close to centers of positive charge (6). The sites are not spatially identical: the number of sites of high affinity for alkyl carboxylates is smaller than the number of such sites for alkyl sulfates (3) and competitive binding studies have shown that only three of the four high affinity sites for deoxycholate are also high affinity sites for alkyl sulfates (7). A few of the sites can bind aliphatic alcohols (3) and the nonionic detergent Triton X-100 (7), but the association constants are considerably smaller than for anionic ligands with comparable alkyl chains. Since the polar head groups of these neutral ligands contain oxygen, it is possible that an ion-dipole interaction at the centers of positive charge contributes to their affinity. Pure hydrocarbons, which have no possible means for polar interaction, do not bind strongly to any site. Binding of butane and pentane has been observed (31), but the data suggest that what is measured is a very low level of saturation of a large number of weak sites.

In light of these data, the small number of binding sites for C14NMe3+ and their relatively small binding constants (Table I) are not unexpected. It is in fact somewhat surprising to find binding sites for cationic detergents at all, since polar interactions presumably contribute to the binding free energy; the cationic detergents, unlike the neutral amphiphiles mentioned above, cannot utilize the same polar sites as the anionic amphiphiles. Assuming that the same hydrophobic patches are involved, the results suggest that four of the sites are close to a glutamate or aspartate residue, perhaps located at the opposite edge of the hydrophobic area from the center of positive charge.

Apart from serum albumin, few common globular proteins have binding sites for any long chain alkyl derivatives. β-Lactoglobulin has one high affinity site that does not depend on any polar interaction: it can bind pure hydrocarbon and C12OSO3− (1). It was not investigated in this study, but very likely would bind cationic detergents as well. Native ovalbumin has no high affinity sites for anionic detergents (32) or for Triton X-100 (7), and only very weak affinity for deoxycholate (7). No binding of cationic detergent to the native protein was detected in this study.

Cooperative Binding and Denaturation—The cooperative association between C14NMe3+ and proteins at high detergent concentration is qualitatively similar to the corresponding process for C12OSO3−. Virtually all proteins seem capable of undergoing the reaction, the accompanying changes in optical rotation and circular dichroism are almost identical for the two detergents, and the resulting complexes have nearly the same hydrodynamic properties. However, the detergent concentrations at which the cooperative transition occurs are significantly different, being higher for the cationic detergent: e.g. the transition of reduced proteins with sodium dodecyl sulfate is complete at an unbound detergent concentration of 8 × 10−4 M (4), whereas the binding of C14NMe3+ is only about 0.1 g per g of protein at the same concentration. The difference is particularly significant because C14NMe3+ has a longer alkyl chain and a correspondingly greater intrinsic hydrophobicity than sodium dodecyl sulfate, and its cmc is lower than that of sodium dodecyl sulfate.

One consequence of the difference between the two detergents is that we were prevented by the onset of micelle formation from reaching saturation of the protein with detergent. Fig. 5 and similar data at pH 8.1 indicate that the saturation level of binding for reduced proteins is at least 1 g of C14NMe3+ per g of protein, but we cannot come closer than this to a comparison with the 1.4 g of detergent per g of protein saturation level observed with C12OSO3−. The formation of complexes at a lower saturation level, that are stable over a limited range of detergent concentration in the protein-C12OSO3− system (4), was not observed with the cationic detergent. It should be noted, however, that the change in optical rotation seen in Fig. 6 is not synchronous with the extent of binding, being almost complete at a binding level of 0.2 g per g. This and the relatively flat slope of the cooperative part of the binding isotherm would be consistent with a state of equilibrium between two or more complexes of disulfide bonds, greatly diminishes the ellipticity to a value much closer to that of the model cystine derivative.

Very different behavior has been found for proteins that have near ultraviolet ellipticity bands that may be attributed to aromatic amino acids. These bands (in several proteins) disappear completely in the cooperative transition induced by either C14NMe3+ or C12OSO3−. Fig. 8 illustrates this result for bovine carbonic anhydrase, a protein devoid of cystine or cysteine, so that no ambiguity in the assignment of the spectrum is possible.

Hydrodynamic Measurements—Foster and Yang (29) have observed an increase in viscosity for bovine serum albumin and ovalbumin in dodecyltrimethylbenzylammonium chloride that is comparable to the viscosity increase in C12OSO3−. Few et al. (9) observed a concentration-dependent viscosity increase for bovine serum albumin in C12NMe3+. We have used Stokes radii (Rg) determined by gel chromatography as a measure of the increase in the extent of the molecular domain. The data shown in Fig. 9 were all obtained with reduced and carboxymethylated proteins, except that bovine carbonic anhydrase did not require this treatment since it contains neither cysteine nor cystine. Measurements with proteins containing disulfide bonds were not made, since irregular results, depending on the location of disulfide bonds, are expected (30).

Fig. 9 shows that Rg is a regular function of the molecular weight of the poly peptide chains, numerical values falling intermediate between those observed for the two types of complexes between reduced proteins and C12OSO3−, containing 0.4 and 1.4 g of detergent per g of protein, respectively. The slope of the plot (d log Rg/d log M) is 0.70, essentially the same as for the C12OSO3− complexes, and indicates that the conformation of reduced proteins at the maximal attainable level in C14NMe3+ can be approximated as rod-like, with some flexibility, or as elongated prolate ellipsoids of revolution.

Interaction between reduced C14NMe3+ and various proteins provided an opportunity to explore the hydrophobic surface of these proteins. The relative binding of C14NMe3+ to ovalbumin in C12OSO3− is about 0.1 g per g, whereas the binding of C14NMe3+ to bovine serum albumin is at least 1 g per g. The data also suggest that what is measured is a very low level of saturation of a large number of weak sites.

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Fig. 9. Stokes radius as a function of molecular weight, with disulfide bonds reduced and carboxymethylated. Results for C14NMe3+ (○) represent the maximal binding level. Results for C12OSO3− (Ref. 5) are given for two levels of binding, 1.4 g per g (△) and 0.4 g per g (□). Proteins used in the present study were lysozyme, immunoglobulin L and II chains, bovine carbonic anhydrase B, ovalbumin, bovine serum albumin, and conalbumin.
different composition, which coexist over a fairly wide range of detergent concentration.

The difference in the critical concentration for the cooperative binding cannot be explained in the same way as the failure to observe cooperative binding at all for nonionic detergents (7). The latter have lower cmc values than ionic detergents of the same alkyl chain length because the repulsion between head groups is less. If the head group has little effect on the affinity for the denatured protein, the competitive advantage between association with the protein and self-association to form micelles can thus shift toward micelle formation when an ionic head group is replaced by a nonionic one. Replacement of one ionic head group by another has, however, no significant effect on the cmc, and the cmc of C14NMe3+ is about the same as the cmc of C12OSO3−. The cationic detergent is nevertheless less effective than either C12OSO3− or C14OSO3− in promoting the cooperative transition.

To seek a possible explanation for this difference, we consider the cooperative transition in terms of the reaction scheme presented previously (2, 21):

\[
\begin{align*}
N + K_D \rightleftharpoons D \\
NL_m \rightleftharpoons DL_m
\end{align*}
\]

In this scheme N represents the native state of the protein without bound detergent or other ligand (designated by L), and D represents a particular denatured state, again without bound ligand. For discussion of the present data D is the rod-like conformation adopted in detergent solutions. The equilibrium constant \(D/N\) is represented by \(K_D\) and its value in the absence of bound ligand is of course very small. \(NL_m\) represents complexes formed by binding of the ligand to the native state, such as we have observed for serum albumin, and their existence leads to stabilization of the native state; \(DL_m\) represents complexes formed by binding of ligand to the denatured conformation, and their existence promotes transition to the denatured state when the ligand concentration is sufficiently high. To consider the difference between the cooperative binding of C14NMe3+ and C12OSO3−, we can ignore the formation \(NL_m\), since this complex does not contribute to the process except for unreduced bovine serum albumin, whereas the observed difference applies to all of the proteins we have examined. Furthermore, the spectral and hydrodynamic data indicate that the denatured state D formed by the anionic and cationic detergents is very similar so that it is unlikely that the difference between them can be ascribed to a difference in \(K_D\).

We are thus forced to conclude that the observed over all ad vantage of the anionic detergent arises from the actual association of the detergents with the denatured form of the protein, and that it must be taken to indicate that the head group plays a significant role in the mechanism of binding. Since the cooperative transition in C12OSO3− occurs at the same detergent concentration for a large number of proteins, and the same is true for C14NMe3+ for the two proteins for which data were obtained, the involvement of the head group must not depend on specific features of the amino acid sequence or other aspects of protein structure. A reasonable possibility is that the detergent cannot be bound as a uniform layer over the entire surface of the extended polypeptide chains because it would be subject to electrostatic repulsion at positions where side chains of like charge projects from the surface. Thus detergent ions, while primarily attracted to the protein by hydrophobic interactions, would tend to cluster about side chains of opposite charge. (The number of bound detergent ions is much larger than can be accounted for on the basis of one detergent per charged side chain.) The association of an anionic detergent would then be favored because arginyl and lysyl side chains project further from the surface and contribute more CH2 groups for incorporation in a cluster as compared to the glutamyl and aspartyl side chains that would be involved in the binding of a cationic detergent.

This explanation suggests that the difference we have observed between C14NMe3+ and C12OSO3− should be a general one, applicable to all cationic and anionic detergents with long alkyl chains.

Use of Cationic Detergents for Analytical Gel Electrophoresis—The method of determining the molecular weights of polypeptide chains by gel electrophoresis in C12OSO3− (33, 34) depends upon the fact that nearly all polypeptide chains in this solvent have the same charge density (i.e. bind the same amount of detergent on a weight basis) and a Stokes radius that depends only on molecular weight (5). Since the transition from the native state to the denatured polypeptide chains is completed well below the cmc of the detergent, the method allows some leeway in the intrinsic stability of the native protein toward denaturation.

It has been suggested that gel electrophoresis in cationic detergents might be as effective a procedure as electrophoresis in C12OSO3− (10, 11), but the results of this study indicate that this is not so. Under the conditions of our experiments we were not able to achieve saturation of the detergent-protein complexes, i.e. the results indicate that polypeptide chains in the presence of an excess of C14NMe3+ will generally be poised at a steep portion of the cooperative binding isotherm. Under these circumstances small individual differences between proteins can produce much larger variations in charge density than is possible in the case of C12OSO3−, and this would increase the probability of individual deviations from the dependence of electrophoretic mobility on molecular weight alone.

Use of Cationic Detergents for Solubilization of Membrane Proteins—It can be expected that extraction of membranes with an excess of cationic detergent will usually lead to solubilization of proteins in a denatured state similar to that obtained when C12OSO3− is used. The successful use of cationic detergents for solubilization of rhodopsin in what appears to be an undenatured state (12, 13) is almost certainly an example of the sensitivity of the conformational transition to variations in the stability of the native state. In rhodopsin the native state is stabilized by the interaction with retinal, and this shifts the detergent activity (i.e. monomer concentration) required for the transition to a higher value that apparently cannot be reached because of the onset of micelle formation. We have observed in this laboratory that the apoprotein opsin, produced by dissociation of retinal from rhodopsin, is not stable toward denaturation by C14NMe3+.

Note Added in Proof—The major part of the apparent decrease in binding observed at the highest detergent concentrations in Fig. 4 may be attributable to the Donnan effect. The influence

1 The effect of pH on the cooperative transition, which is in the opposite direction for C12OSO3− and C14NMe3+, presumably also results in large part from the association of detergent with the denatured protein. One of us speculated in a previous paper (21) that the pH effect on the transition induced by C12OSO3− might arise from a dependence of \(K_D\) on pH. This must now be considered unlikely, since, if the denatured state formed for the two detergents is the same or nearly the same, the pH dependence of \(K_D\) would be in the same direction for the two detergents.
of this effect on the distribution of the ligand in equilibrium dialysis is magnified when the concentration of the ligand ion is increased relative to that of unreactive electrolyte, even though the total electrolyte concentration also increases. We thank Dr. Neal Robinson for drawing our attention to this.

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J. Biol. Chem. 1974, 249:4452-4459.

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