# THE INTERACTION OF TRITON X-100 WITH SOLUBLE PROTEINS: POSSIBLE IMPLICATIONS FOR THE TRANSPORT OF PROTEINS ACROSS MEMBRANES

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SUMMARY

Dodecyl sulfate complexes of two soluble proteins, serum albumin and fumarase, have been "renatured" with large excesses of the nonionic detergent Triton X-100. The resulting complexes, essentially free of dodecyl sulfate, differ in their sedimentation properties relative to the native protein and in their interaction with Triton X-100. Albumin molecules refold to a form binding only very small amounts of Triton and have a sedimentation coefficient similar to that of the non-denatured protein. On the other hand, refolded fumarase molecules have a lower sedimentation coefficient than that of the native enzyme and bind up to 1.06 mg of Triton/mg protein. It is postulated that the fumarase molecule has been turned "inside-out" by the dodecyl sulfate/Triton treatment, and the implications of such large conformational changes for protein transport across membranes are discussed.

The interaction of proteins with a mild detergent, Triton X-100, has been shown to reflect the lipid interactions of these molecules. Intrinsic membrane proteins bind up to their own weight in this detergent, while soluble and extrinsic membrane proteins, which do not interact with the hydrophobic part of the lipid bilayer, bind no detergent (1-3). For proteins that do not exclusively interact with detergent micelles, one can quantitate the hydrophobic surface area by determining the amount of bound Triton X-100 (1).

To extend the usefulness of detergent binding as a criterion of protein lipid interaction, I decided to test whether proteins denatured in sodium dodecyl sulfate maintained similar interactions with Triton X-100 as the native proteins. If this were so, one would be able to obtain information on the lipid interactions of proteins separated from complex mixtures by dodecyl sulfate gel electrophoresis, or could compare the lipid interactions of the individual subunits of membrane proteins which can only be obtained in the denatured state. It has already been demonstrated that the erythrocyte anion transport protein (band III), an intrinsic membrane protein, could be refolded from its dodecyl sulfate complex into a form that still bound Triton X-100 (1).

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The purpose of this work was to show whether typical soluble proteins maintained their lack of interaction with Triton X-100 after denaturation in dodecyl sulfate and refolding in large excesses of Triton.

In this report, I present evidence that the Triton interactions of at least one soluble protein are markedly altered after dodecyl sulfate/Triton treatment. The results suggest that the folding of this protein can proceed by at least two paths to give molecules that differ greatly in the arrangement of their hydrophobic and hydrophilic residues.

## METHODS

Materials: [$^3$H]Triton X-100, a gift of Dr. William Lyman (Rohm & Haas, Philadelphia, PA), was prepared as described (1). Sodium dodecyl [$^{35}$S]sulfate (4.3 mCi/mmol) from New England Nuclear was diluted 9 fold with non-isotopically labeled dodecyl sulfate (Sigma, 95%) to a concentration of 10 mg/ml.

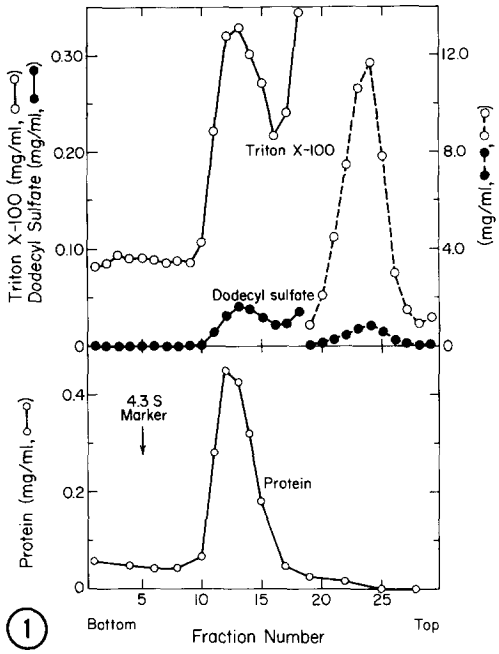
Pig heart fumarase was obtained from Sigma as a crystalline suspension in 3.2 M ammonium sulfate at a specific activity of 360 units/mg protein. Bovine serum albumin (crystallized and lyophilized), rabbit muscle pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase, and aldolase were also obtained from Sigma. Pig heart mitochondrial malate dehydrogenase was a Boehringer Mannheim product. Proteins were recovered from ammonium sulfate suspensions by centrifugation, and their concentrations determined by ultraviolet absorption, using the extinction coefficients given in Ref. 4.

Interaction of Triton X-100 with Denatured Proteins: Soluble proteins were incubated with a 2.5 fold weight excess of sodium dodecyl [$^{35}$S]sulfate in the presence of 2-mercaptoethanol at an ionic strength of 70 mM. Under these conditions, full denaturation and maximal levels of dodecyl sulfate binding have been observed for a number of proteins (5). These complexes were then incubated with a 25 fold weight excess (in terms of protein) of [$^3$H]Triton X-100. These amounts correspond to a molar ratio of Triton to dodecyl sulfate of 4.6 and absolute concentrations of these detergents of 31.4 and 6.9 mM.

Protein complexes were separated from mixed dodecyl sulfate/Triton micelles and monomers by centrifugation in gradients containing various amounts of [$^3$H] Triton X-100. The amount of each detergent bound to the protein was determined by double isotope counting. Control experiments performed in the absence of protein indicated that both detergents comigrated near the top of the gradient. Specific experimental details are given in the figure legends and in Ref. 1.

## RESULTS

Complexes formed by adding large amounts of Triton X-100 to dodecyl sulfate-denatured proteins were analyzed after separation from detergent micelles and monomers as described above. Fig. 1 shows the result of an experiment performed with fumarase. Control experiments demonstrated that the native enzyme (without dodecyl sulfate treatment) bound less than 0.014 mg of Triton X-100 per mg protein at micellar concentrations of detergent. However, when the protein was first denatured with dodecyl sulfate, a very large amount of Triton X-100 was found to be bound to the modified protein. The protein-Triton complex was quantitatively recovered in a sharp band that was well resolved from the mixed



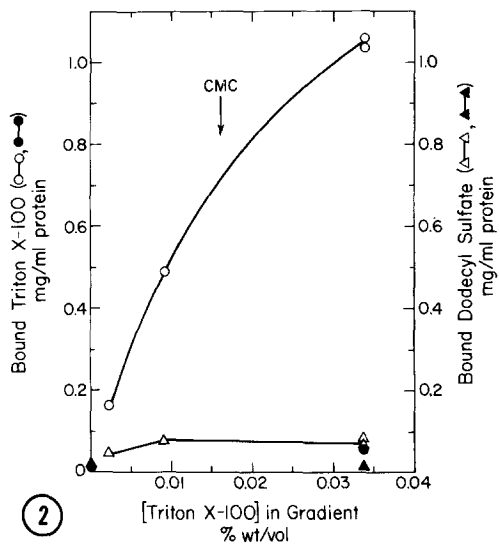




Fig. 1. Triton X-100 binding to denatured fumarase. Pig heart enzyme (0.57 mg) was incubated with dodecyl [$^{35}$S]sulfate (1.5 mg, 1046 cpm/μg) in 16 mM 2-mercaptoethanol and 50 mM Tris sulfate pH 7.8 for 3 min at 100° and then for 2 h at 37° (total volume is 0.35 ml). A large excess of [$^{3}$H]Triton X-100 (16.5 mg in 0.18 ml, 51.8 cpm/μg) was then added and the incubation continued for 60 min at 37°. An aliquot (0.18 ml) of this mixture was centrifuged in a 4 ml 5 to 20% sucrose gradient at 4° for 23 h at 60,000 rpm in a SB-405 rotor of an IEC B-60 ultracentrifuge. The gradient contained 0.0089% [$^{3}$H]Triton X-100, 1 mM 2-mercaptoethanol, and 50 mM Tris sulfate pH 7.8. Fractions were collected from the bottom of the tube and analyzed for protein by alkaline ninhydrin assay, and for the two detergents by counting 0.03 ml aliquots in 2.5 ml Aquasol (New England Nuclear) on a Beckman LS-230 scintillation counter and correcting for cross-talk (less than 5%) between the [$^{35}$S] and narrow [$^{3}$H] channels. Note that the radioactivity in the micelle fraction at the top of the gradient (fractions 19-29) is displayed at 1/40th the scale of the other fractions. The peak position of a 4.3 S marker (malate dehydrogenase) is shown by an arrow. This enzyme (2.5 μg) was added to the sample immediately before centrifugation, and was enzymatically assayed.

Fig. 2. Determination of bound Triton X-100 and dodecyl sulfate to fumarase (open circles and squares) and serum albumin (closed circles and squares) after protein-dodecyl sulfate complexes were treated with a large excess of Triton X-100. The resulting protein-detergent complexes were separated from the mixed micelles of Triton X-100 and dodecyl sulfate by sucrose gradient centrifugation as shown in Fig. 1. The amount of each bound detergent for the 4 S albumin complex and the 3 S fumarase complex is shown for various experiments where the gradients contained amounts of Triton above and below the critical micelle concentration (CMC).

micelles at the top of the gradient. Only a small residual amount of bound dodecyl sulfate (0.01 to 0.07 mg/mg protein) was found to be associated with the complex. At concentrations of Triton X-100 above the critical micelle concentration in the gradient, binding of up to 1.06 mg/mg protein was obtained (Fig. 2).

When the same experiments were performed with bovine serum albumin, a different picture was obtained. Although the "renatured" protein was again recovered in a sharp band on the gradient, only very small amounts of either Triton X-100 or dodecyl sulfate were found to be associated with the modified protein (Fig. 2). Preliminary experiments were also performed with malate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, and aldolase. With the exception of malate dehydrogenase, these proteins did not remain in solution after Triton X-100 was added to the dodecyl sulfate-protein complex.

The sedimentation coefficients of the Triton-refolded fumarase and albumin molecules were estimated from the gradient position of the protein peak relative to that of a marker enzyme (malate dehydrogenase, 4.3 S) which was added to the sample immediately before centrifugation (6). The calculated $s_{20,w}$ of 4.0 S for Triton/dodecyl sulfate treated albumin is very similar to that of the native protein, and this value is consistent with the refolding of the dodecyl sulfate-albumin complex into a monomer with a conformation similar to that of the native protein. For the Triton-refolded fumarase molecule, the calculated sedimentation coefficient is 2.84 S. This value is very different from that of the native protein, a tetramer of 48,500 dalton subunits, of 9.1 S. Although the presence of bound detergent will make this calculated $s_{20,w}$ somewhat lower than the true value (1,6), one can calculate that the corrected value of 3.0 S indicates that either the fumarase molecule has not refolded to a compact conformation or that it has not regained its quaternary structure. This sedimentation coefficient is consistent with a rather globular monomer of fumarase, or with more extended structures for higher aggregates.

## DISCUSSION

It was originally hoped that proteins "renatured" from dodecyl sulfate complexes by Triton X-100 would maintain (at least qualitatively) the same kind of interaction with Triton X-100 as the native protein. Although this does seem to be the case for the red blood cell anion transport protein (1) and for albumin, the results of this study show that at least one typically soluble protein, fumarase, which does not interact with Triton X-100 in the native state, can bind up to its own weight in detergent after denaturation. Thus, the interaction of this refolded molecule with Triton X-100 cannot be

distinguished from that of an intrinsic membrane protein and it is therefore not possible to deduce the detergent interactions of native proteins from the interactions of dodecyl sulfate denatured molecules.

For albumin, the removal of bound dodecyl sulfate proceeds without massive Triton X-100 binding, and the sedimentation coefficient of the renatured complex is similar to that of the native molecule. In this way, the results of this type of procedure follow those obtained when dodecyl sulfate-protein complexes were renatured via urea treatment and passage over ion exchange resins (7)

The situation for Triton-"renatured" fumarase is quite different. The binding of 1.06 mg Triton X-100/mg protein corresponds to 81 bound molecules of Triton per polypeptide chain of 48,500 daltons. Furthermore, there appear to be individual binding sites for detergent monomers because binding occurs below the critical micelle concentration of Triton X-100; there is no sharp transition in the binding curve at the critical micelle concentration (Fig. 2).

Table I shows a proposed classification for two types of interaction of membrane proteins with Triton X-100. One class of these proteins interacts only with detergent micelles, presumably through a hydrophobic "tail" of amino acid residues. Another class appears to interact with detergent monomers at hydrophobic surfaces on the molecules (1). By the criteria given in this table, especially in the binding of detergent below the critical micelle concentration, Triton-renatured fumarase has the same binding interactions as the Class II intrinsic membrane proteins, and would appear to have a large exposed hydrophobic surface. This result suggests that fumarase has folded in the presence of Triton X-100 to give a conformation markedly different from that of the active enzyme. It seems likely that this new conformation results from a reversal, or turning inside out, of the native structure where the hydrophobic groups are located in the interior of the molecule and the hydrophilic groups are on the exterior.

It is not clear whether such a dodecyl sulfate-mediated conformational transition of fumarase to a molecule with a hydrophobic surface has any physiological significance. Nevertheless, it is clear that the mitochondrial matrix proteins (like fumarase) are synthesized on cytoplasmic ribosomes and must be transported across both the outer and inner mitochondrial membranes (12,13). The mechanism for the transfer of such normally hydrophilic proteins across the hydrophobic lipid bilayers is not understood; however, the existence of at least two stable conformational states of fumarase, one hydrophilic and one hydrophobic, might help explain the transfer. *In vivo*, one might postulate that membrane lipids initiate the same kind of conformational state in newly synthesized fumarase that Triton X-100 performs with the denatured enzyme to allow passage of this molecule across the bilayer.

TABLE I

TWO MODES OF PROTEIN INTERACTIONS WITH MEMBRANES AND TRITON X-100

| | MODE OF INTERACTION WITH LIPID BILAYER | MODE OF INTERACTION WITH DETERGENT (ABOVE CMC) | CHARACTERISTIC FEATURES | | EXAMPLES[a] |
|---|---|---|---|---|---|
| | | | BEHAVIOR IN SUB-MICELLAR DETERGENT CONCENTRATIONS | AMINO ACID SEQUENCE | |
| CLASS I PROTEINS | Hydrophobic "tail" intercalates into lipid bilayer | Hydrophobic "tail" inserts into detergent micelle or forms nucleation site for new micelle | Sharp transition in detergent binding curve at cmc; no binding below cmc; protein aggregation | Extended sequences containing hydrophobic residues | RBC Major Sialoglycoprotein (1)<br>Cytochrome $b_5$ (8)<br>$\gamma$-glutamyl transpeptidase (9)<br>Phage fd coat protein (10)<br>CRM 45 fragment of diphtheria toxin (11) |
| CLASS II PROTEINS | Lipid monomers attach to specific hydrophobic binding sites on protein surface | Detergent monomers bind to hydrophobic surface, replacing bound lipid molecules | No sharp transition in binding curve at cmc; detergent bound below cmc | Extended hydrophobic sequences not necessary; hydrophobic surface can form by correct positioning of side chains | Serum albumin (1,3)<br>Low density lipoprotein (1)<br>RBC anion transport protein (1) |

[a] References given in parenthesis

Studies of the interaction of Triton X-100 with diphtheria toxin and its fragments have recently been described which indicate that a similar situation to that of fumarase described here may exist (11). This toxin, a portion of which must transverse the membrane bilayer for activity, does not bind Triton in the native state. However, when first denatured with dodecyl sulfate, the toxin binds large amounts of Triton X-100 below the critical micelle concentration.

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