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Ca^{2+}-ATPase–DETERGENT INTERACTIONS

A Good Model for Protein-Lipid Interactions

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The Ca^{2+}-ATPase from sarcoplasmic reticulum has been demonstrated to have an obligatory requirement for bound amphiphiles, either phospholipids or detergents (1, 2). The results from several different approaches have suggested that 30–40 molecules of phospholipid are associated with the ATPase in native or reconstituted membranes (3, 4). However, it has not been possible to determine the affinity for phospholipid, or to observe directly the interaction of phospholipid monomers with the protein because of aggregation of phospholipid molecules at very low concentrations. In the present report, the interaction of detergent molecules below the critical micelle concentration (c.m.c) with delipidated Ca^{2+}-ATPase is described. In addition, the effect of temperature on detergent-ATPase interactions is investigated.

METHODS

Binding of [^{3}H]Triton X-100 to the delipidated ATPase (3, 5) was observed on a Sepharose 6B column equilibrated with 0.01 M TES buffer, pH 7.5, containing 0.1 M KCl, 2.74 M glycerol, 1.0 mM dithiothreitol, and the desired concentration of [^{3}H]Triton X-100 at 6°C (6). Binding was calculated from the difference in radioactivity between column fractions containing ATPase protein, and fractions free of protein. Ca^{2+}-ATPase activity was determined by a coupled assay (4). Steady-state anisotropy of 1,6-diphenyl-1,3,5-hextriene (DPH) was estimated using a Glan polarizer in conjunction with an Aminco-Bowman spectrophotofluorometer with a water-jacketed cell compartment.

RESULTS AND DISCUSSION

Binding of [^{3}H]Triton X-100 to the delipidated ATPase occurred in two steps. First, from 1×10^{-5}M to 12×10^{-5}M Triton, binding of the detergent occurred at 35–40 equivalent sites. Near the c.m.c (20×10^{-5}M), cooperative binding of an additional 70 mol of Triton was observed. About half of the Ca^{2+}-ATPase activity was recovered during the noncooperative binding process and the remaining activity was observed after the cooperative binding was complete. The value of 35–40 Triton molecules bound per ATPase molecule is in good agreement with the result reported for the number of phospholipid molecules associated with the Ca^{2+}-ATPase in native or reconstituted membranes (3). The cooperative binding of additional Triton molecules is probably associated with micelle formation and incorporation of the ATPase into detergent-protein aggregates. The affinity of the ATPase for Triton molecules below the cmc is probably at least several hundred-fold lower than the affinity of the ATPase for phospholipid molecules, based on the 300-fold greater molar ratio of Triton needed for complete reactivation of the delipidated ATPase as compared with phosphatidylcholine (2, 5). However, the affinity constant reported here may not apply directly to the binding of Triton to individual ATPase molecules because of the highly aggregated state of the protein under these conditions. These results support the hypothesis that distinct binding sites occur on the Ca^{2+}-ATPase for phospholipid molecules in the native membrane and that these sites must be occupied for the ATPase to be active. However, the degree of immobilization of the lipid cannot be predicted from these results since no kinetic measurements were attempted.

It is well documented that a break occurs in the Arrhenius plot of ATPase activity in native membranes near 20°C (3, 5) and that this transition temperature can be changed by substituting different lipids in reconstitution experiments (3). However, the nature of the lipid-protein interactions that affect the enzymatic behavior of the ATPase is a subject of controversy. In the present study, the temperature dependence of ATPase activity has been determined for the delipidated ATPase in three different detergents as shown in Table I. The observation that the Arrhenius plots exhibit different discontinuity temperatures in different detergents suggests that the micelle environment can affect this property of the ATPase; this differs from the initial conclusion of Dean and Tanford (6). Table I also shows the results of steady-state fluorescence polarization measurements of DPH in the same three detergents. These results suggest that some type of structural perturbation is occurring in the detergents at the same temperature at which ATPase activity is affected. This alteration can also be detected by paranaric acid fluorescence and differential scanning calorimetry

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TABLE I
EFFECT OF TEMPERATURE ON ATPase ACTIVITY AND DPH ANISOTROPY

Detergent	Temperature of discontinuity of Arrhenius plot of ATPase activity (°C)	Temperature of discontinuity in DPH anisotropy (°C)
$C_{12}E_8$	20	18
Triton X-100	25	25
Tween 80	16, 30	16

(5). The structural perturbation possibly represents a rearrangement of polar head groups or a change in the state of aggregation of the micelle since it is highly unlikely that phase transitions occur in the hydrocarbon interior of these micelles (7). Thus, as has been observed for phospholipids, temperature-dependent changes in detergent micelles affect the activity of the ATPase.

The results presented here demonstrate that the study of detergent protein interaction can lend additional insight into the details of protein-lipid interaction. The effects of nonionic detergents on the Ca^{2+}-ATPase from sarcoplasmic reticulum are quite similar to those of phospholipids, and the relatively high c.m.c of detergents allows for direct observation of amphiphile monomer interactions with the protein.

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INTERDIGITATION OF FATTY ACID CHAINS OF DIPALMITOYLPHOSPHATIDYLGLYCEROL DUE TO INTERCALATION OF MYELIN BASIC PROTEIN

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The basic protein (BP) of myelin is water soluble and binds electrostatically to acidic lipids but has several hydrophobic segments which may intercalate partway into the bilayer. This conclusion is based on the perturbing effects of the protein on the bilayer (1–3) as well as evidence for sequestration of some parts of the protein by the lipid (4, 5). Differential scanning calorimetry (DSC) and fatty acid spin labels were used to investigate the dependence of this interaction on the phase state of dipalmitoylphosphatidylglycerol (DPPG).

RESULTS

Intercalation of BP into DPPG decreases the temperature, T_c, and enthalpy, ΔH, of its phase transition (2). The thermograms of DPPG and the DPPG-BP complex for two different sets of samples are shown in Fig. 1. The first set (Fig. 1 *b*, *d–f*) contain 52.4% BP by weight and the second set (Fig. 1 *g*, *j–m*) contains 48% BP. ΔH measurements were made for the second set and are given in the figure caption. BP has a greater effect on the T_c and ΔH on the second and repeated heating scans (Fig. 1 *e* and *k*) than on the first (Fig. 1 *b* and *g*) compared to the pure lipid (Fig. 1 *a* and *h*). This suggests that greater intercalation occurs in the liquid-crystalline phase than in the gel phase (2, 3). Consequently BP has an even greater effect on the cooling scan (Fig. 1 *d* and *j*) compared to the cooling scan of the pure lipid (Fig. 1 *c* and *i*). However, if the sample is reheated from a higher temperature (12–17°C rather than from below 12°C as above) the relative effect on the heating scan (Fig. 1 *f* and *l*) is similar to that on the cooling scan. Most of the thermogram consists of an endothermic peak at 30–35°C. A similar endothermic peak begins in the reheating scans shown in Fig. 1 *e* and *k* but is not completed. An exothermic process occurs