Tulinsky, A., Mavridis, I., & Mann, R. F. (1978) *J. Biol. Chem. 253*, 1074–1078.
Vandlen, R. L., & Tulinsky, A. (1971) *Acta Crystallogr., Sect. B 27*, 437–442.
Vandlen, R. L., & Tulinsky, A. (1973a) *Acta Crystallogr., Sect. B 29*, 1309.
Vandlen, R. L., & Tulinsky, A. (1973b) *Biochemistry 12*, 4193–4200.
Venkatachalam, C. M., Price, B. J., & Krimm, S. (1974) *Macromolecules 7*, 212.
Venkatachalam, C. M., Price, B. J., & Krimm, S. (1975) *Biopolymers 14*, 1121.
Wallace, R. A., Kurtz, A. N., & Niemann, C. (1963) *Biochemistry 2*, 824–836.
White, D. N. J., & Morrow, C. (1977) *Comput. Chem. 1*, 225–233.
Zerner, B., & Bender, M. L. (1964) *J. Am. Chem. Soc. 86*, 3669–3674.
Zerner, B., Bond, R. P. M., & Bender, M. L. (1964) *J. Am. Chem. Soc. 86*, 3674–3679.

Interactions between Sarcoplasmic Reticulum Calcium Adenosinetriphosphatase and Nonionic Detergents†

William L. Dean* and Carolyn P. Suárez

ABSTRACT: The interaction of Triton X-100 and other nonionic detergents with a delipidated preparation of the Ca^{2+}ATPase from sarcoplasmic reticulum has been studied. Binding of radiolabeled Triton X-100 was determined by column chromatography at 6 °C, and two classes of binding sites were observed. Below the critical micelle concentration (cmc), binding of Triton occurred at 35–40 equivalent sites on the delipidated ATPase with a binding constant of 2.7×10^4 M^{-1}. Near the cmc cooperative binding of an additional 70 molecules of the detergent was observed. The binding of monomeric Triton X-100 below the cmc was associated with a parallel activation of over half of the ATPase activity, and the remainder of the activity was recovered after the detergent concentration was increased to the cmc. The ability to reactivate ATPase activity was more dependent on the polar poly(oxyethylene) portion of nonionic detergents than on the hydrocarbon portion. Generalizing for all amphiphiles, these results suggest that there are discrete binding sites on the Ca^{2+}ATPase for phospholipid molecules in the native membrane and that the polar head groups of phospholipids interact more strongly with the protein than the hydrophobic acyl chains. Perturbations in micelle structure were observed for several nonionic detergents by measurement of *cis*-parinaric acid fluorescence and differential scanning calorimetry, and discontinuities in Arrhenius plots occurred at the transition temperature of the detergent used for reactivation of ATPase activity. It is concluded that both the physical state of the micelle and the intrinsic behavior of the ATPase polypeptide affect the temperature dependence of ATPase activity.

The Ca^{2+}ATPase[1] from sarcoplasmic reticulum has been demonstrated to have an obligatory requirement for bound amphiphiles. In the absence of amphiphiles the ATPase cannot hydrolyze ATP, but when the appropriate phospholipids or detergents are bound, ATP hydrolysis occurs in the presence of Ca^{2+} (Martonosi, 1968; Warren et al., 1974; Dean & Tanford, 1977). The results from several different approaches have suggested that ~30 mol of phospholipid is associated with the ATPase in a phospholipid bilayer (Warren et al., 1974; Hesketh et al., 1976; le Maire et al., 1976). Since phospholipid molecules aggregate to form bilayers at very low concentrations (e.g., 4.6×10^{-10} M for dipalmitoylphosphatidylcholine; Smith & Tanford, 1972), it has not been possible to determine the affinity of phospholipid molecules for the ATPase. In fact, there is a controversy as to how the kinetics of phospholipid association is related to ATPase activity, and it has been suggested that lipid molecules may exchange during the time scale of the enzymatic turnover (Rice et al., 1979).

Because detergents have much higher cmc's than phospholipids (Tanford, 1973), they are well suited for the study of protein–amphiphile interactions. In the present report the affinity of [^{3}H]Triton X-100 for the delipidated Ca^{2+}ATPase (Dean & Tanford, 1977) is determined by a column chromatographic technique (Hummel & Dreyer, 1962). Since Triton X-100 effectively supports ATPase activity, the relationship between detergent monomer binding and activation of ATPase activity can be observed directly. Furthermore, since a wide variety of detergents of differing structure are commercially available, it is possible to assess the relative importance of the polar and nonpolar portions of detergents for supporting ATPase activity.

Another aspect of amphiphile–ATPase interaction that can be readily probed with nonionic detergents is the effect of the physical state of the amphiphile on ATPase activity. Several laboratories have reported discontinuities in Arrhenius plots of ATPase activity near 20 °C for the Ca^{2+}ATPase in its native membrane (Inesi et al., 1973; Lee et al., 1974; Dean & Tanford, 1978; Moore et al., 1978). This behavior has been attributed to a liquid-crystalline transition of the lipid closely associated with the ATPase, since the bulk lipid displays a transition at a much lower temperature (Hesketh et al., 1976; Davis et al., 1976). However, a delipidated preparation reactivated with the nonionic detergent $C_{12}E_8$ also exhibited a discontinuity near 20 °C, and the authors concluded that

† From the Department of Biochemistry, University of Louisville School of Medicine, Louisville, Kentucky 40292. *Received August 18, 1980.* This work was supported by a grant from the Muscular Dystrophy Association.

[1] Abbreviations used: $C_{12}E_8$, dodecyl octaethylene glycol monoether; cmc, critical micelle concentration; ATP, adenosine 5′-triphosphate; Ca^{2+}ATPase, calcium adenosinetriphosphatase; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

0006-2960/81/0420-1743$01.25/0

this behavior was a result of temperature-dependent changes in the protein unrelated to lipid–protein interactions (Dean & Tanford, 1978). In the present report it is shown that $C_{12}E_8$ exhibits a structural perturbation near 20 °C and that the temperature dependence of ATPase activity of the delipidated protein in detergents is indeed related to the physical characteristics of the detergent micelle.

Materials and Methods

Tritons X-100, X-165, X-305, X-102, and N-101, poly(oxyethylene) 9-lauryl ether, poly(oxyethylene) 10-tridecyl ether, and poly(oxyethylene) 10-lauryl ether were purchased from Sigma Chemical Co. Zwittergents 3-8, 3-12, and 3-16 were obtained from Calbiochem-Behring Corp. [^{3}H]Triton X-100 was a product of New England Nuclear. *cis*-Parinaric acid was purchased from Molecular Probes, Inc. The source and preparation of all other materials used in the present study are the same as those reported earlier (le Maire et al., 1976; Dean & Tanford, 1977).

Sarcoplasmic reticulum was isolated from rabbits by the method of Eletr & Inesi (1972) followed by sucrose density gradient centrifugation (Dean & Gray, 1980). Purified ATPase vesicles were prepared according to method 2 of Meissner et al. (1973). Delipidation of the purified ATPase was carried out as previously described (Dean & Tanford, 1978). ATPase activity, protein, and phospholipid were determined as described earlier (Dean & Tanford, 1978). In the temperature-dependence studies, assay mixtures were preincubated at the desired temperature for 3–5 min prior to the addition of ATPase, and the temperature of the reaction mixture was determined immediately after completion of the assay with a telethermometer (Yellow Springs Instruments). The assays for temperature-dependence experiments were carried out with 10 mM ATP, and it was determined that maximal velocity was achieved at this ATP concentration at all temperatures studied. Assays of the delipidated ATPase in various detergents were carried out at 30 °C with 5 mM ATP. A series of concentrations was used for each detergent so that the maximal stimulation could be determined as described earlier (Dean & Tanford, 1978).

[^{3}H]Triton X-100 binding was determined at 6 °C on a Sepharose 6B column (1 × 30 cm) equilibrated with 0.01 M TES buffer, pH 7.5, containing 0.1 M KCl, 2.74 M glycerol, 1 mM dithiothreitol, and the desired concentration of Triton X-100 with a specific activity of 0.124 mCi/mmol of Triton X-100. Delipidated ATPase (0.1–0.3 mg) was dissolved in 0.3 mL of the column buffer containing 8.06×10^{-4} M Triton X-100 at the same specific activity as the detergent on the column. The sample was applied to the column, and chromatography was carried out with a flow rate of 0.3–0.5 mL/min. Fractions were collected, weighed, and assayed for ATPase activity, radioactivity, and protein concentration. Because of the presence of dithiothreitol, glycerol, and Triton X-100, all of which interfere with the Lowry protein assay (Lowry et al., 1951), it was more convenient to use the Bio-Rad protein assay (Bio-Rad Laboratories) calibrated with delipidated ATPase [see Bradford (1976)]. Binding was determined by measuring the difference between the radioactivity eluting with the ATPase and that in the column buffer (Hummel & Dreyer, 1962). The specific activity of the ATPase after chromatography was generally the same as the activity before chromatography and varied from 10–20 μmol min^{-1} mg^{-1} at 37 °C when assayed in 1.6×10^{-4} M Triton X-100, and the phospholipid content was 3–5 mol/mol of ATPase.

Fluorescence measurements were performed on an Aminco-Bowman spectrophotofluorometer with a ratio photometer in the ratio mode. The temperature of the solution in the cell was maintained by a water-jacketed cell holder, and the temperature was determined with a Yellow Springs telethermometer. Differential scanning calorimetry was performed on a Perkin-Elmer DSC-1 by using water and $C_{12}E_8$, which melts at 27 °C, as temperature standards. Scanning calorimetry was carried out at a rate of 2.5°/min with 0.1 M solutions of the detergents in water.

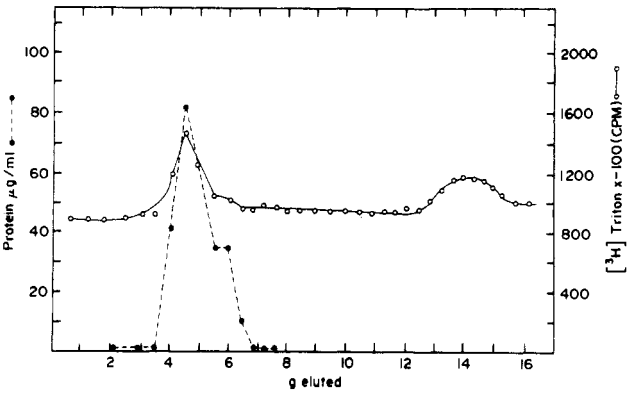

FIGURE 1: Typical gel chromatogram of delipidated ATPase in Triton X-100 on Sepharose 6B. Delipidated ATPase (0.15 mg) was suspended in 0.3 mL of column buffer containing 8.0×10^{-4} M [^{3}H]Triton X-100. The column was equilibrated and chromatography was carried out as described under Materials and Methods with buffer containing 1.6×10^{-4} M [^{3}H]Triton X-100.

Results

Triton X-100 Binding. Binding of [^{3}H]Triton X-100 to the reversibly delipidated ATPase was carried out on a Sepharose 6B column as described under Materials and Methods. A typical elution profile of ATPase and detergent is shown in Figure 1. Because of the low detergent concentration (1.6×10^{-4} M in Figure 1), the ATPase is aggregated and elutes at the void volume of the column at all concentrations of Triton X-100 used in these experiments. The eluted ATPase is accompanied by bound Triton X-100 as shown by the increase in radioactivity. The excess detergent that was applied with the ATPase elutes at the same position as dithiothreitol, as would be expected for monomeric Triton X-100 on this column.

The results of binding and activity measurements at 6 °C are shown in Figure 2. Both binding and ATPase activity increase in hyperbolic fashion from 1×10^{-5}–15×10^{-5} M Triton X-100. Near the cmc for Triton X-100, a cooperative increase in Triton binding occurs as expected for insertion of the ATPase into a micelle. The cmc for Triton X-100 in water is 2.4×10^{-4} M (Kushner & Hubbard, 1954) but is somewhat lower, 2.0×10^{-4} M, in the buffer used for these experiments at 6 °C as determined by fluorescence enhancement of parinaric acid. If the Triton concentration of the sample loaded on the column was at the same concentration as that of the buffer on the column, essentially the same binding results were obtained. However, there was some loss of ATPase on the column, probably a result of the highly aggregated state of the delipidated protein at these low detergent concentrations.

In Figure 3 the binding data from 1×10^{-5}–12×10^{-5} M Triton is plotted according to Scatchard (1949). The line determined by least-squares analysis shows that there are 35–40 equivalent sites on the ATPase molecule for Triton monomers and that the affinity for this amphiphile is 2.7×10^4 M^{-1}. The number of Triton sites is in excellent agreement with the results of Hesketh et al. (1976) for the number of phospholipid molecules bound to the ATPase in a membrane

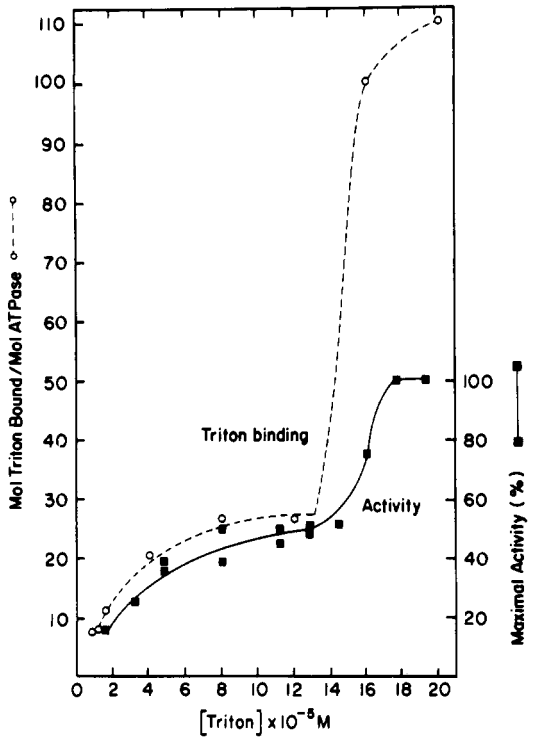

FIGURE 2: Effect of Triton X-100 concentration on binding and activity of the delipidated ATPase. Binding measurements were carried out as shown in Figure 1 and as described under Materials and Methods. Delipidated ATPase was assayed in the specified concentration of Triton X-100 at 6 °C.

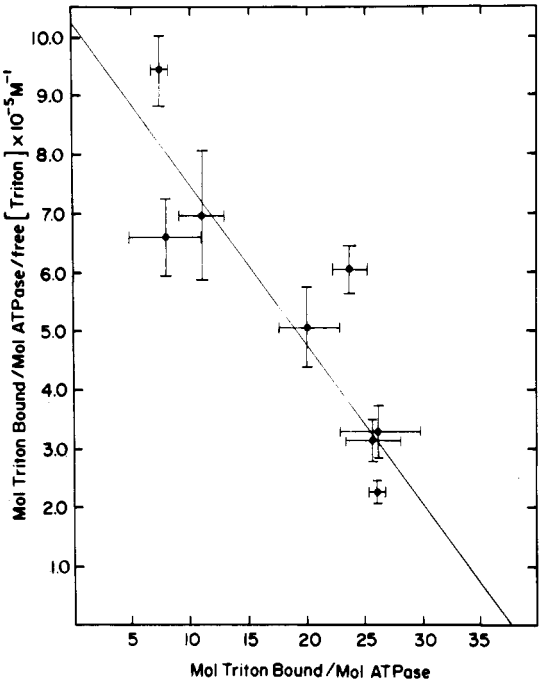

FIGURE 3: Scatchard plot of Triton X-100 binding. Binding data from 1×10^{-5}–12×10^{-5} M Triton X-100 is plotted according to Scatchard (1949). The error bars show the standard deviation from the mean of the binding data obtained from a single chromatography experiment for several points on the major peak of ATPase elution from the Sepharose 6B column. Since the amount of Triton bound appears in both axes of the plot, error in both dimensions in shown.

as determined by lipid titration and from lipid spin-label studies. Thus Triton molecules are probably binding to the same sites as phospholipid molecules. It is likely that the ATPase has much greater affinity for phospholipids than nonionic detergents as shown by the ~300-fold greater molar ratio needed for complete reactivation of delipidated ATPase

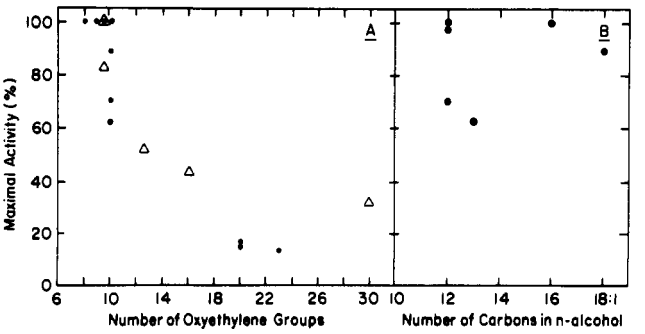

FIGURE 4: Effect of nonionic detergent structure on reactivation of delipidated ATPase. (A) The number of oxyethylene groups was varied while keeping the rest of the detergent molecule approximately unchanged. (○) represents detergents of the Brij series containing a straight-chain alcohol in an ether linkage with a straight poly(oxyethylene) chain. (△) represents detergents of the Triton series which contain a tertiary octylphenyl alcohol in ether linkage with a linear poly(oxyethylene) chain. The detergents used were $C_{12}E_8$ [8], poly(oxyethylene) 9-lauryl ether [9], Lubrol PX [9.5], poly(oxyethylene) 10-tridecyl ether [10], Brij 56 [10], Brij 96 [10], Brij 58 [20], Brij 98 [20], Brij 35 [23], Triton X-100 [9.5], Triton N101 [9.5], Triton X-102 [12.5], Triton X-165 [16], and Triton X-305 [30]. (Numbers in brackets refer to the number of oxyethylene groups.) (B) The number of oxyethylene groups was held constant while the straight-chain alcohol portion was varied. The detergents used were $C_{12}E_8$ [12], poly(oxyethylene) 9-lauryl ether [12], poly(oxyethylene) 10-tridecyl ether [13], Brij 56 [16], and Brij 96 [18:1]. (Numbers in brackets refer to the number of carbons in the alcohol.)

by $C_{12}E_8$ as compared with phosphatidylcholine (Dean & Tanford, 1977).

Structural Requirements for ATPase Activation. In earlier work (Dean & Tanford, 1978) it was shown that a variety of detergents can reactivate the delipidated ATPase. In the present report this approach has been extended to demonstrate the effect of the polar oxyethylene and hydrophobic hydrocarbon portions of detergents on ATPase activity. Figure 4A shows the effect of increasing the number of oxyethylene units while keeping the hydrophobic portion of nonionic detergents approximately constant. For both the Triton and Brij series of nonionic detergents, increasing the length of the poly(oxyethylene) portion of the amphiphile reduces the ability of the molecule to reactivate the delipidated ATPase. In contrast, Figure 4B demonstrates that the length and degree of saturation of the hydrocarbon portion of detergent molecules do not appear to be a determinant for the ability of detergents to activate the ATPase. This result is in agreement with the recent work of Rice et al. (1979), who concluded by using NMR techniques that the Ca^{2+}ATPase interacts more strongly with phospholipid head groups than with the fatty acyl chains of phospholipid molecules. However, results for the zwitterionic detergents zwittergents suggest that the binding interaction may be more complex when the head group is charged. Three detergents with the zwitterionic head group 3-(*N*,*N*-dimethylammonio)-1-propanesulfonate were tested. The 8 and 12 carbon chain zwittergents (3-8 and 3-12) did not support significant ATPase activity, whereas a 16-carbon derivative (3-16) was able to reactivate nearly completely.

Temperature Dependence of ATPase Activity. Sklar et al. (1975) described the use of naturally occurring polyunsaturated fatty acids such as parinaric acid for probing membrane properties. These fluorescent molecules partition into the hydrophobic interior of bilayers and are very sensitive monitors of the phase transition of membrane lipids. In the present report *cis*-parinaric acid is used as a probe of nonionic detergent micelles. A plot of relative fluorescence vs. temperature for *cis*-parinaric acid in aqueous buffer or pentane is linear from 10 to 35 °C (data not shown). However, when $C_{12}E_8$

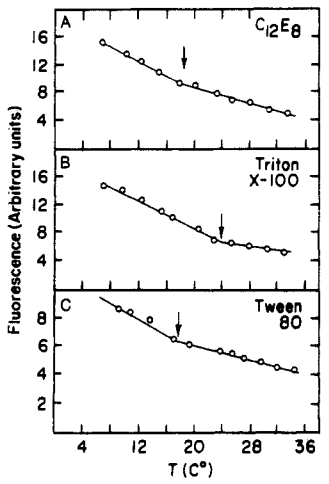

FIGURE 5: Effect of nonionic detergents on parinaric acid fluorescence. *cis*-Parinaric acid was added to a solution containing 0.1 M TES buffer, pH 7.5, 2.74 M glycerol, and 0.1 M KCl to give a final concentration of 4.0×10^{-6} M. The solution also contained the specified detergent at a concentration of 1.0 g/L (1.6×10^{-3} M Triton X-100, 1.9×10^{-3} M $C_{12}E_8$, and 9.2×10^{-4} M Tween 80). Excitation was at 315 nm and emission was recorded at 410 nm.

was added to the buffer above its cmc, a break was observed in the plot of fluorescence vs. temperature at 19 °C as shown in Figure 5A. This break in the plot may represent a rearrangement in polar head groups or other structural perturbations.[2] Phase changes have been detected in micelles of other detergents by fluorescence techniques including cetyltrimethylammonium bromide (37 °C), tetramethylammonium palmitate (33 °C), and cetylbetaine (23 °C) (Shinitzky, 1974). Since the perturbation for $C_{12}E_8$ occurs at the same temperature as the break in the Arrhenius plot published previously (Dean & Tanford, 1978), two other detergents capable of supporting ATPase activity were tested as shown in the other two panels of Figure 5. Triton X-100 exhibited a discontinuity at 25.1 °C and Tween 80 at 16.8 °C. The results of differential scanning calorimetry were in good agreement with this interpretation of the fluorescence data. All three detergents exhibited transitions near the temperatures predicted in Figure 5. $C_{12}E_8$ showed a transition at 18 °C, Triton X-100 at 27 °C, and Tween-80 at 14 °C. Arrhenius plots in Triton X-100 and Tween 80 were constructed as shown in Figure 6. The break in the plot for Triton X-100 occurs at ~25 °C, in good agreement with the structural transition temperature, and similarly with Tween 80 a break occurs at 16 °C. An additional break occurs at ~29 °C in Tween 80 which does not correspond with a change in the fluorescence of parinaric acid in this detergent. This result has not been observed with other amphiphiles but may suggest that temperature-dependent changes in the protein can occur in the absence of a change in the amphiphile environment.

Discussion

The studies presented in this paper demonstrate that the binding of amphiphile monomers at 35–40 specific sites on the Ca^{2+}ATPase is directly related to the maintenance of the protein in its native conformation. Below the cmc of Triton X-100 the binding and ATPase activation curves are parallel (Figure 2), although complete reactivation of the delipidated ATPase does not occur until the protein is inserted in a micelle. By extension of this result to membranes, it seems likely that phospholipid molecules are bound to these specific sites on the

[2] It is unlikely that there is a phase transition of the dodecyl chains of $C_{12}E_8$ (Tanford, 1973).

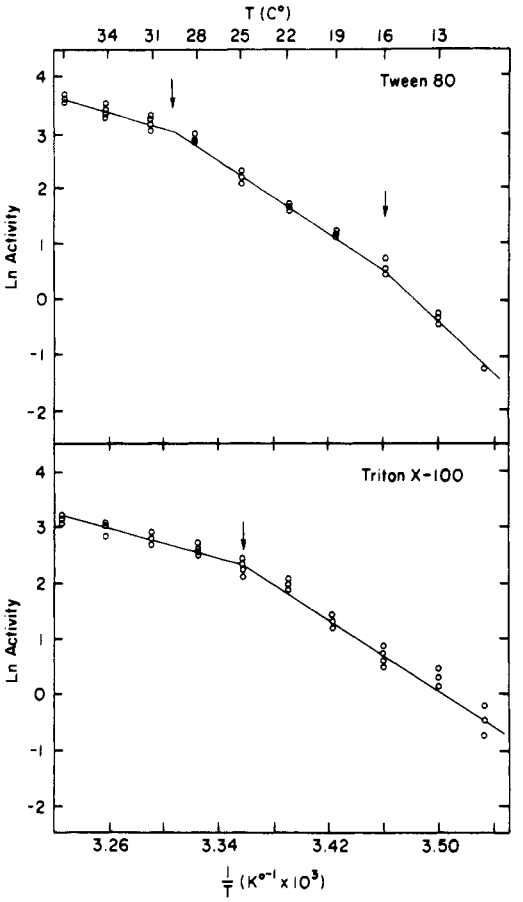

FIGURE 6: Temperature dependence of ATP hydrolysis for delipidated ATPase in Triton X-100 and Tween 80. ATPase activity was determined at the specified temperatures for delipidated ATPase in the presence of either Triton X-100 (1.6×10^{-4} M) or Tween 80 (4.6×10^{-3}M), both of which gave maximal stimulation of ATPase activity.

ATPase with an affinity that may be several orders of magnitude greater than the affinity observed for Triton X-100 based on the relative abilities of nonionic detergents and phospholipids to reactivate the delipidated ATPase. Evidence that nonionic detergents and phospholipids compete reversibly for the same sites on the ATPase was presented earlier in experiments showing the rebinding of phospholipid to the delipidated ATPase in the presence of $C_{12}E_8$ [see Dean & Tanford (1978), Figure 5].

The results of studies on the relationship between the structure of amphiphiles and their ability to reactivate the delipidated ATPase show that the polar head group of nonionic detergents is the major activity determinant, which is in agreement with results obtained for phospholipids with the Ca^{2+}ATPase (Rice et al., 1979) and the Na, K-ATPase (Brotherus et al., 1980). It seems likely that these polar amphiphile–ATPase interactions occur at the binding sites occupied by Triton molecules below the cmc, since these sites are associated with the initial activation of ATPase activity.

The second class of amphiphile sites on the ATPase, those associated with cooperative interactions of the ATPase with detergent molecules that result in insertion of the ATPase into a micelle, also appear to be important for the full expression of ATPase activity. For both $C_{12}E_8$ (Dean & Tanford, 1978) and Triton X-100, the ATPase is not fully reactivated until the concentration of detergent is at the cmc. The binding interactions at these sites are probably more hydrophobic in nature and represent interactions of the protein or previously bound amphiphiles with the hydrocarbon interior of the micelle. Thus the ATPase shows a combination of binding

properties: binding of monomeric detergent to several sites on the protein below the cmc as observed for bovine serum albumin (Makino et al., 1973) and cooperative binding of large amounts of detergent near the cmc as observed for cytochrome b_5 (Robinson & Tanford, 1975).

The effect of the physical state of nonionic detergent micelles on the ATPase activity of the delipidated ATPase is quite striking. The discontinuity in the Arrhenius plot of ATPase activity coincides with the temperature of the structural perturbation of the nonionic detergent used for reactivation (Figure 6). Thus the original conclusion reached from studies on the delipidated ATPase in $C_{12}E_8$ (Dean & Tanford, 1978) that the change in the Arrhenius activation energy is completely intrinsic to the ATPase polypeptide was incorrect. However, the temperature dependence of the ATPase is not solely a function of the state of the micelle as shown by a second discontinuity of the Arrhenius plot derived from studies in Tween 80. The conclusion of Moore et al. (1978) that the temperature dependence of Ca^{2+}ATPase activity is a function of both lipid–protein interactions and changes intrinsic to the protein alone is probably correct.

In conclusion, the work presented here shows that there are indeed specific amphiphile binding sites on the Ca^{2+}ATPase. These sites may be distributed on the ATPase so as to form an amphiphile annulus although other configurations are possible. Of course the measurements reported here are thermodynamic and cannot be applied to the arguments concerning the kinetics of phospholipid–ATPase interactions in bilayers (Hesketh et al., 1976; Rice et al., 1979).

Acknowledgments

We are greatly indebted to Ellen Ford for typing the manuscript and to Dr. Samuel L. Cooke, Jr., Department of Chemistry, University of Louisville, for the use of his differential scanning calorimeter and for his expertise. We also acknowledge the critical review of Dr. Charles Tanford, Duke University.

References

Bradford, M. (1976) *Anal. Biochem. 72*, 248.

Brotherus, J. R., Jost, P. C., Griffith, O. H., Keana, J. F., & Hokin, L. E. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 272.

Davis, D. G., Inesi, G., & Gulik-Kryzywicki, T. (1976) *Biochemistry 15*, 1271.

Dean, W. L., & Tanford, C. (1977) *J. Biol. Chem. 252*, 3551.

Dean, W. L., & Tanford, C. (1978) *Biochemistry 17*, 1683.

Dean, W. L., & Gray, R. D. (1980) *J. Biol. Chem. 255*, 7514.

Eletr, S., & Inesi, G. (1972) *Biochim. Biophys. Acta 282*, 174.

Hesketh, T. R., Smith, G. A., Houslay, M. D., McGill, K. A., Birdsall, N. J. M., Metcalf, J. C., & Warren, J. B. (1976) *Biochemistry 15*, 4145.

Hummel, J. P., & Dreyer, W. J. (1962) *Biochim. Biophys. Acta 63*, 530.

Inesi, G., Millman, M., & Eletr, S. (1973) *J. Mol. Biol. 81*, 483.

Kushner, L. M., & Hubbard, W. D. (1954) *J. Phys. Chem. 58*, 1163.

Lee, A. G., Birdsall, N. J. M., Metcalfe, J. C., Toon, P. A., & Warren, G. B. (1974) *Biochemistry 13*, 3699.

le Maire, M., Moller, J. V., & Tanford, C. (1976) *Biochemistry 15*, 2336.

Lowry, O. H., Rosebrough, N. J., Farr, A. C., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.

Makino, S., Reynolds, J. A., & Tanford, C. (1973) *J. Biol. Chem. 248*, 4926.

Martonosi, A., Donley, J., & Haplin, R. A. (1968) *J. Biol. Chem. 243*, 61.

Meissner, G., Conner, G. E., & Fleischer, S. (1973) *Biochim. Biophys. Acta 298*, 246.

Moore, B. M., Lentz, B. R., & Meissner, G. (1978) *Biochemistry 17*, 5248.

Rice, D. M., Meadows, M. D., Scheinman, A. O., Goni, F. M., Gomez-Fernandez, J. C., Moscarello, M. A., Chapman, D., & Oldfield, E. (1979) *Biochemistry 18*, 5893.

Robinson, N. C., & Tanford, C. (1975) *Biochemistry 14*, 369.

Scatchard, G. (1949) *Ann. N.Y. Acad. Sci. 51*, 660.

Shinitzky, M. (1974) *Isr. J. Chem. 12*, 879.

Sklar, L. A., Hudson, B. S., & Simoni, R. D. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1649.

Smith, R., & Tanford, C. (1972) *J. Mol. Biol. 67*, 75.

Tanford, C. (1973) *The Hydrophobic Effect*, Wiley, New York.

Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., & Metcalfe, J. C. (1974) *Biochemistry 13*, 5501.