Davis, B. J. (1964), *Ann. N.Y. Acad. Sci. 121,* 404.
Fisher, L. M., Albery, W. J., and Knowles, J. R. (1976), *Biochemistry 15,* 5621.
Horecker, B. L., Hurwitz, J., and Weissbach, A. (1958), *Biochem. Prep. 6,* 83.
Kornberg, H. L., and Lascelles, J. (1960), *J. Gen. Microbiol. 23,* 511.
Lorimer, G. H., Andrews, T. J., and Tolbert, N. E. (1973), *Biochemistry 12,* 18.
McCurry, S. D., and Tolbert, N. E. (1977), *J. Biol. Chem. 252,* 8344.
McFadden, B. A., and Tabita, F. R. (1974), *Biosystems 6,* 93.
Model, P., Ponticorvo, L., and Rittenberg, D. (1968), *Biochemistry 7,* 1339.
Norton, T., Welch, M., and Hartman, F. C. (1975), *J. Biol. Chem. 250,* 8062.
Orr, G. A., and Knowles, J. R. (1974), *Biochem. J. 141,* 721.
Paech, C., Ryan, F. J., and Tolbert, N. E. (1977), *Arch. Biochem. Biophys. 179,* 279.
Rabin, B. R., and Trown, P. W. (1964a), *Proc. Natl. Acad. Sci. U.S.A. 51,* 497.
Rabin, B. R., and Trown, P. W. (1964b), *Nature (London) 202,* 1290.
Racker, E. (1962), *Methods Enzymol. 5,* 266.
Rutner, A. C. (1970), *Biochem. Biophys. Res. Commun. 39,* 923.
Schloss, J. V., and Hartman, F. C. (1977), *Biochem. Biophys. Res. Commun. 75,* 320.
Sugiyama, T., Akazawa, T., Nakayama, N., and Tanaka, Y. (1968), *Arch. Biochem. Biophys. 125,* 107.
Tabita, F. R., and McFadden, B. A. (1974a), *J. Biol. Chem. 249,* 3453.
Tabita, F. R., and McFadden, B. A. (1974b), *J. Biol. Chem. 249,* 3459.
Takabe, T., and Akazawa, T. (1975), *Arch. Biochem. Biophys. 169,* 686.
Trown, P. W., and Rabin, B. R. (1964), *Proc. Natl. Acad. Sci. U.S.A. 52,* 88.
Whitman, W. B., and Tabita, F. R. (1976), *Biochem. Biophys. Res. Commun. 71,* 1034.
Whitman, W. B., and Tabita, F. R. (1978), *Biochemistry 17,* 1282.
Wishnick, M., and Lane, M. D. (1969), *J. Biol. Chem. 244,* 55.

Denaturation of the Tryptic Fragments of the Calcium(II) Adenosine Triphosphatase from Sarcoplasmic Reticulum by Guanidinium Hydrochloride†

Lawrence J. Rizzolo* and Charles Tanford

ABSTRACT: Primary and secondary fragments of the Ca^{2+}-adenosine triphosphatase from sarcoplasmic reticulum are resistant to complete denaturation by guanidinium hydrochloride, a property characteristic of many intrinsic membrane proteins. None of the fragments display a single cooperative transition from ordered structure to random coil suggesting each fragment contains several domains of differing resistance to guanidinium hydrochloride denaturation. The data suggest that the native enzyme has at least three membrane-embedded domains, with an externally accessible link between each.

The structure and mechanism of ATP-dependent cation transport proteins are not fully understood. Considerable progress has been made with the Ca^{2+}-ATPase[1] of sarcotrypsin (Ikemoto et al., 1971; Inesi & Scales, 1974; Thorley-Lawson & Green, 1975; Stewart et al., 1976). The enzyme is composed of a polypeptide of 119 000 molecular weight (Rizzolo et al., 1976), which contains the ATP and Ca^{2+} binding sites (Degani & Boyer, 1973; Stewart et al., 1976). Enzyme preparations also contain a small polypeptide, termed proteolipid by MacLennan et al. (1972), which may be involved in enzyme activity (Racker & Eytan, 1975). Trypsin cleaves the ATPase into two equal size fragments, I and II, whose molecular weights determined by sedimentation equilibrium correspond to one half the weight of the intact chain (Rizzolo et al., 1976). Fragment I can be further cleaved by trypsin to form two fragments of approximately 35 000 (I_A) and 25 000 (I_B) molecular weight, determined by $NaDodSO_4$ gel electrophoresis (Stewart et al., 1976; Thorley-Lawson & Green, 1975). Neither cleavage has any affect on ATP hydrolysis or calcium translocation activities (Inesi & Scales, 1974; Stewart & MacLennan, 1974).

Thorley-Lawson & Green (1975) found that the amino acid composition of each fragment is very similar to that of the intact ATPase. Using the formulation of Capaldi & Vanderkooi (1972), the percentage of hydrophilic residues for each fragment is only slightly less than the average for water-soluble proteins. The use of antibodies against the various fragments suggests that fragment I is in large part exposed to solution, whereas fragments II and I_B are only slightly exposed (Stewart et al., 1976). This is consistent with the preferential iodination of fragment I by Thorley-Lawson & Green (1973). These data

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[1] Abbreviations used: ATPase, adenosine triphosphatase; ATP, adenosine triphosphate; $NaDodSO_4$, sodium dodecyl sulfate; GdmCl, guanidinium hydrochloride; CD, circular dichroism.

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suggest fragment II is buried in the membrane, while fragment I is exposed to solution. Digestion with the nonspecific protease preparation Nagarse (Yamanaka & Deamer, 1976) readily digested the ATPase to peptides of approximately 10 000 molecular weight, but it is not clear how much of the ATPase had been cleaved away and how much had been protected by the membrane. The number of peptides resulting from the digestion was not established.

We have observed that the ATPase and fragments I and II are resistant to complete denaturation by 6 M guanidinium hydrochloride; aggregation occurred in this normally dissociating solvent and optical measurements demonstrated retention of folded regions of the polypeptide backbone (Rizzolo et al., 1976). This phenomenon is rare among water soluble proteins (Tanford, 1968). On the other hand, resistance to denaturation by guanidinium hydrochloride may be a characteristic of most intrinsic membrane protein (Steck & Yu, 1973) and several membrane-embedded proteins which have been sequenced exhibit this property (Nozaki et al., 1978; Tajima et al., 1976; Grefrath & Reynolds, 1974). The resistance to GdmCl denaturation might be caused by clusters of hydrophobic residues characteristic of a membrane-embedded polypeptide. It is known that segments of polyleucine and polyphenylalanine that are incorporated into water-soluble polypeptides are not denatured by GdmCl (Auer & Doty, 1966).

In the present study, we examine in detail the behavior of each fragment in GdmCl. All four tryptic fragments of the Ca^{2+}-ATPase resist complete denaturation to random coil suggesting that each fragment contains hydrophobic regions and that the polypeptide backbone must therefore weave in and out of the membrane at least three times.

Experimental Section

Materials. Sodium dodecyl sulfate was purchased from B.D.H. Chemicals Ltd. and was >99% homogeneous by gas-chromatographic analysis of the aliphatic alcohol produced by hydrolysis. Sodium dodecyl [^{35}S]sulfate was purchased from Amersham/Searle. Guanidinium hydrochloride, UltraPure grade, was obtained from Schwarz/Mann and met the criteria for purity established by Wong et al. (1971). Iodoacetamide and iodoacetic acid (Sigma Corp.) were recrystallized from hexane. TPCK-trypsin was purchased from Worthington Biochemical Corp. *p*-Nitrophenyl *p*-guanidinobenzoate was synthesized according to the method of Chase & Shaw (1970).

Sarcoplasmic reticulum was prepared as described by Meissner et al. (1973), and purified ATPase vesicles were obtained by method 2 of Meissner et al. (1973).

Preparation of Fragments. Trypsin cleavage was carried out essentially as described by previous investigators (Ikemoto et al., 1971; Thorley-Lawson & Green, 1973; Inesi & Scales, 1974). Primary tryptic fragments (I and II) were prepared as follows. ATPase vesicle suspensions containing 5–40 mg of protein, at concentrations of 1–5 mg/mL, were incubated for 5 min in 20 mM Tris-maleate, pH 7.0, 0.1 M NaCl, 1 M sucrose, and trypsin at a 200:1 protein/trypsin weight ratio. The reaction was stopped by adding an excess of *p*-nitrophenyl *p*-guanidinobenzoate. The product was dissolved in 0.1 M $NaDodSO_4$, 0.13 M β-mercaptoethanol, and heated for 2 min at 100 °C. The fragments were purified by $NaDodSO_4$–hydroxylapatite chromatography followed by gel filtration chromatography as described previously (Rizzolo et al., 1976).

Secondary cleavage products (I_A and I_B) were prepared under similar conditions using a 5:1 protein/trypsin weight ratio with a 20-min incubation. The vesicles were washed several times with 20 mM Tris-maleate buffer, pH 7.0, 0.1 M NaCl to remove trypsin; and then dissolved in $NaDodSO_4$, as above. The sample was chromatographed on Bio-Gel A-1.5m in 0.05 M phosphate buffer, pH 7.0, containing 3.47 mM $NaDodSO_4$.

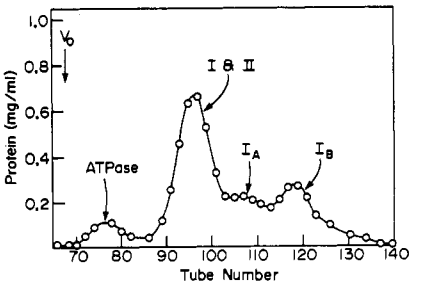

FIGURE 1: Gel filtration of fragments I_A and I_B in $NaDodSO_4$. ATPase vesicles (30 mg of protein) were cleaved with trypsin at a 5:1 trypsin/protein weight ratio, dissolved in $NaDodSO_4$ and loaded on a Bio-Gel A-1.5m column (1.5 × 180 cm) and eluted with 0.05 M phosphate buffer, pH 7.0, in 3.47 mM $NaDodSO_4$. The purity of fragment I_A (tubes 106–112 were used) and fragment I_B (116–125 were used) is indicated by the gels in Figure 2.

Removal of Sodium Dodecyl Sulfate. The disulfide bonds of the fragments were reduced with diothiothreitol and the free sulfhydryls were alkylated with either iodoacetic acid or iodoacetamide. The $NaDodSO_4$ was removed by dialysis against 0.01 M phosphate buffer, which contained suspended Dowex 1-X8 anion-exchange resin to prevent buildup of the $NaDodSO_4$ concentration outside the dialysis bag. The GdmCl concentration was adjusted by dialysis against the appropriate GdmCl solution or by the addition of solid crystals. The samples were then reduced and alkylated again, and the low molecular weight impurities were removed by dialysis or chromatography.

Protein Concentration. Protein concentrations were determined by the method of Lowry et al. (1951) using BSA as the standard and were corrected for the 1.2-fold higher color yield reported for the ATPase by Hardwicke & Green (1974). Concentrations in GdmCl solutions were determined using a calculated specific extinction coefficient at 280 nm (Edelhoch, 1967) based on the aromatic amino acid content of the fragments (Thorley-Lawson & Green, 1975). We obtained $E_{1\%}^{1cm}$ = 12.4 for the intact ATPase. Values for fragments I (11.4), II (14.6), I_A (12.2), and I_B (10.4) were also calculated.

Other Methods. Circular dichroism measurements were carried out using a Jobin Yvon Dichrographe III. Sedimentation equilibrium measurements were made with a Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner. $NaDodSO_4$ gel electrophoresis was performed by the method of Weber & Osborn (1969).

Results

Purification of Fragments I_A and I_B. Gel filtration in $NaDodSO_4$ on Bio-Gel A-1.5m was sufficient to purify the secondary fragments in one step. The elution pattern presented in Figure 1 shows fragments I and II coelute ahead of the secondary fragments. Fragment I_B overlaps the $NaDodSO_4$–phospholipid mixed micelle peak. The mixed micelles were removed by further chromatography on Sephacryl S-200. The purity of the fragments is shown in Figure 2.

Removal of Sodium Dodecyl Sulfate. Dialysing the purified fragments in $NaDodSO_4$ against a low ionic strength buffer containing suspended anion exchange resin removed greater

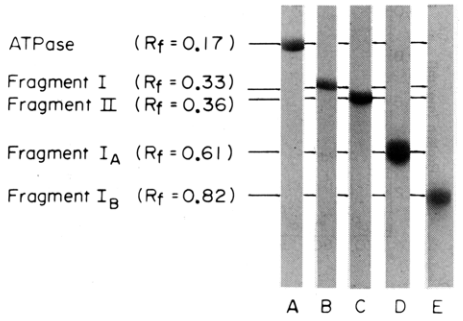

FIGURE 2: Sodium dodecyl sulfate gel electrophoresis. (A) ATPase; (B) fragment I; (C) fragment II; (D) fragment I_A; (E) fragment I_B. Gels A, B, and C were taken from Rizzolo et al. (1976).

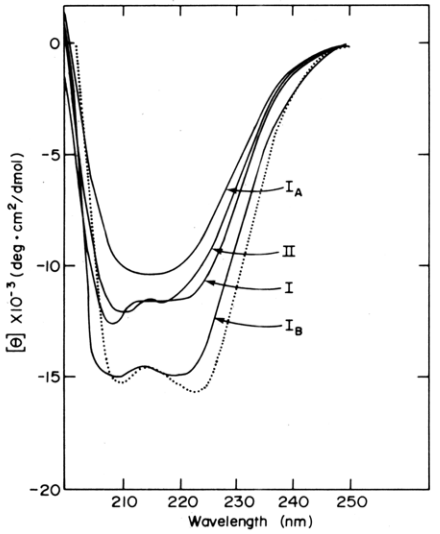

FIGURE 3: Circular dichroism spectra of tryptic fragments in aqueous buffer. The fragments were reduced and alkylated and the $NaDodSO_4$ removed by dialysis vs. 0.01 M phosphate buffer, pH 7.0, and suspended anion-exchange resin. The dotted spectrum of fully active ATPase in nonionic detergent is included for reference (Dean & Tanford, 1978).

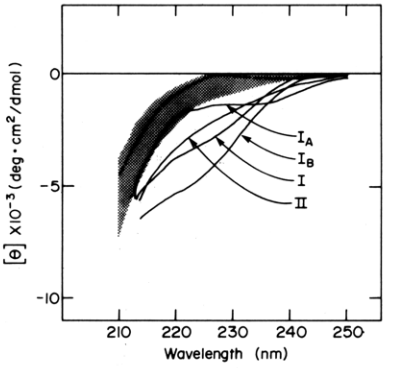

FIGURE 4: Circular dichroism spectra of reduced and alkylated proteins in 8.2 M guanidinium hydrochloride. Identical spectra were obtained after the samples were incubated at room temperature for several weeks. The shaded area shows the range of ellipticities found for nine randomly coiled proteins in 6 M GdmCl (Cortijo et al., 1973). The dashed line is the spectrum of randomly coiled fd coat protein in 7.3 M GdmCl (Nozaki et al., 1978).

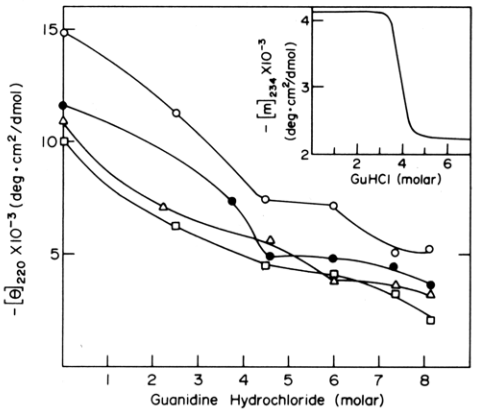

FIGURE 5: Denaturation of reduced and alkylated fragments by guanidinium hydrochloride at room temperature. The molar ellipticity at 220 nm is plotted as a function of GdmCl concentration. Data points represent the average of several experiments. No change in the molar ellipticity was observed after the samples were incubated at room temperature for several weeks. (●) fragment I; (△) fragment II; (□) fragment I_A; (○) fragment I_B. The inset shows the denaturation of lysozyme as followed by the molar rotation at 234 nm as a function of GdmCl concentration (Tanford et al., 1966).

than 99% of the $NaDod^{35}SO_4$. Each fragment was soluble in aqueous buffer in the absence of detergent at protein concentrations less than 0.5 mg/mL, provided the fragment had been reduced and alkylated. The circular dichroism (CD) spectra of the water-soluble fragments are presented in Figure 3. The dotted curve shows the spectrum of fully active ATPase in nonionic detergent solution. Obviously, the fragments have not retained the conformation they had in the native ATPase, as the average of the spectra for fragment I and II would have approximated the spectrum for the native ATPase otherwise. The weighted average of the spectra for subfragments I_A and I_B (assuming molecular weights of 35 000 and 25 000, respectively) roughly approximates the spectrum for fragment I, suggesting the possibility that in this denatured form the subfragments are structurally independent of one another. The spectra were reproducible as long as the protein concentration was maintained below 0.5 mg/mL. Increasing the protein concentration results in precipitation and irreversible conformational changes. Substituting the negatively charged iodoacetic acid for the neutral iodoacetamide as the alkylating agent did not affect the aggregation here or in the studies presented below.

Resistance to Denaturation in Guanidinium Hydrochloride. We have previously shown that fragments I and II are not random coils in 6 M GdmCl (Rizzolo et al., 1976) and have obtained the same result for fragments I_A and I_B. Even in 8.2 M GdmCl only fragment I_A comes close to random coil and the others are far from it (Figure 4). Clearly none of the spectra fell in the range observed in 6 M GdmCl, by Cortijo et al. (1973) for a number of proteins known to be randomly coiled. Included in the figure (dashed line) is the spectrum of randomly coiled fd coat protein, a membrane-embedded peptide.

The progressive change in the conformation of the fragments as a function of GdmCl concentration can be seen in terms of the plot of the molar ellipticity, θ, at 220 nm vs. the GdmCl concentration (Figure 5). None of the fragments displayed the sharp transition exemplified by the curve for lysozyme in the insert, but the transition occurred over a broad range of GdmCl concentration. In 8.2 M GdmCl only fragment I_A approached the range of θ_{220} values expected for a random coil.

Sedimentation equilibrium experiments were performed in 6.0 M GdmCl and curved plots, indicative of considerable aggregation, were obtained. The slope near the middle of the solution column yielded a weight average molecular weight of about 300 000 for the intact ATPase and values of about 140 000 for fragments I and II. Fragments I_A and I_B pelleted to the bottom of the cell at rotor speeds appropriate for de-

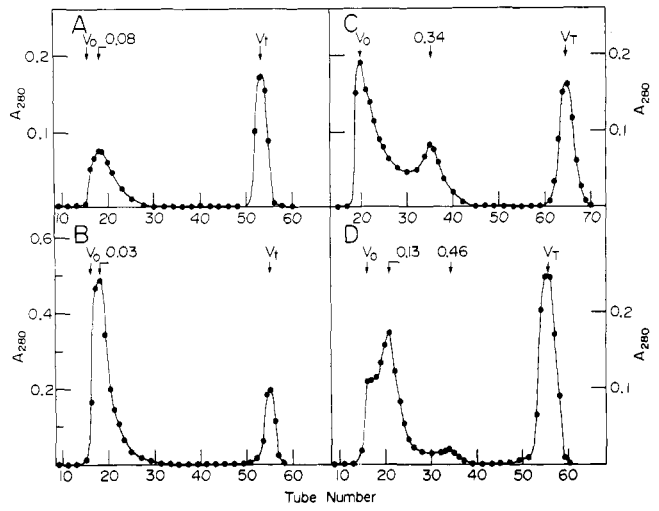

FIGURE 6: Gel filtration of the tryptic fragments in 8.2 M guanidinium hydrochloride. Fragments in saturated GdmCl solution were heated for 15 min at 40 °C and loaded on a Sepharose 4B column (0.8 × 70 cm) which was equilibrated and eluted with 25 mM phosphate buffer, pH 7.0, and 8.2 M GdmCl. The total volume was marked in A-D with dithiothreitol and the void volume was marked in C and D with blue dextran. (A) fragment I; (B) fragment II; (C) fragment I_A; (D) fragment I_B.

termining the monomer molecular weight, indicating very large aggregates. The choice of alkylating agent had no effect.

Gel filtration experiments were performed in 7.3 and 8.2 M GdmCl on a calibrated Sepharose 4B column (Fish et al., 1970). The samples for these experiments were heated at 40 °C in saturating concentrations of GdmCl and then loaded on the column, which was eluted at room temperature with either 7.3 or 8.2 M GdmCl.

In the case of fragments I and II, essentially identical elution patterns were obtained at 7.3 and 8.2 M GdmCl. The elution pattern for the latter concentration is shown in Figure 6. The distribution coefficients (K_d) of 0.03 for fragment I and 0.08 for fragment II were less than the K_d of 0.17 for transferrin (molecular weight 76 000), indicating the fragments had a larger Stokes radius than transferrin and therefore must have been aggregated. The small trailing shoulder for each fragment suggests that a column equilibrated at a higher GdmCl concentration at 40 °C might dissociate the fragments further. The CD spectra for fragments I and II in 8.2 M GdmCl did not fall in the expected range for randomly coiled polypeptide (Figure 4).

In the case of the subfragments I_A and I_B, the elution pattern of the column equilibrated at 8.2 M GdmCl shows some of the sample was dissociated, possibly to monomer units (Figure 6). For each fragment the large void volume peak is blue dextran. Most of fragment I_B elutes near the void volume with the remaining protein spread between this peak and a small peak whose K_d is 0.46. If the protein in the latter peak was randomly coiled, its elution position could be used to estimate the polypeptide molecular weight even if the protein could reversibly associate to form oligomers. Since the monomer species of a reversibly associating system migrates the slowest on a gel filtration column, its elution position is unaffected by the kinetics of the association-dissociation reaction. Unfortunately, a CD spectrum of the tube with a K_d of 0.46 could not be taken because the protein concentration was too low. If one is willing to make the assumption that the protein in this peak was randomly coiled, the molecular weight would be 23 000 which is consistent with the result from $NaDodSO_4$ gel electrophoresis. The CD of the major peak is presented in Figure 4.

Most of fragment I_A eluted with a K_d of 0.34 in 8.2 M GdmCl (Figure 6), while the trailing edge of the blue dextran peak indicated some protein eluted close to the void volume. The CD spectra of the major peak, shown in Figure 4, indicates this fragment might be randomly coiled, in which case the molecular weight of fragment I_A is 37 000. These preliminary estimates of the molecular weights of fragments I_A and I_B sum to equal the molecular weight of fragment I.

Discussion

The intact ATPase and the tryptic fragments are sparingly soluble in water, provided the sulfhydryl groups have been blocked, but prefer the environment of a lipid bilayer or a detergent micelle. The literature supports this conclusion, though the evidence is less compelling for the fragments I_A and I_B (Thorley-Lawson & Green, 1975; Stewart et al., 1976). It is not unreasonable to suspect these polypeptides contain clusters of hydrophobic residues in the amino acid sequence.

It has been noted that resistance of membrane-embedded proteins to complete denaturation by guanidinium hydrochloride might be ascribed to the presence of large clusters of hydrophobic amino acid residues. Tomita and Marchesi (1975) have shown the M,N-glycoprotein of the human erythrocyte contains a hydrophobic segment of 23 amino acid residues, which presumably reside in the membrane, while Grefrath & Reynolds (1974) have observed this protein is aggregated in 6 M GdmCl. Microsomal cytochrome b_5 contains two 8-residue hydrophobic segments in its membrane-embedded domain (Corcoran & Strittmatter, 1977). Tajima et al. (1976) have found that this domain, which can be separated from the rest of the protein by limited trypsin digestion, requires a high concentration of GdmCl for complete denaturation, while the hydrophilic domain behaves like most water-soluble proteins. The coat protein of fd bacteriophage has a single polypeptide chain of 50 amino acid residues containing an uninterrupted sequence of 19 uncharged and predominantly hydrophobic residues (Naskashima & Konigsberg, 1974). Nozaki et al. (1978) observed that succinylation was required to prevent aggregation of the coat protein in 6.0 M GdmCl, 7.3 M GdmCl being required to form monomeric random coils without succinylation. All of the tryptic fragments are exceptionally resistant to GdmCl denaturation supporting the contention that they are membrane embedded. Several features distinguish the behavior of the fragments from the other membrane proteins that have been studied. For the fragments, the transition to random coil is broad and is not complete (except possibly for I_A) at 8.2 M GdmCl. Superficially, the fd coat protein and the hydrophobic fragment of cytochrome b_5 show a single-step transition which is complete by 6.5–7.3 M GdmCl and occurs over a range of ~2–3 M GdmCl (compared with a range of ~1 M for lysozyme). The midpoint of the transition for these proteins is approximately 5 M GdmCl. These polypeptides are smaller than the smallest ATPase fragment and a major fraction of their sequence is membrane embedded. Intact cytochrome b_5 has a two-step transition, the first step being ascribable to the globular hydrophilic domain. The broad range of GdmCl concentration required for denaturation of the ATPase fragments suggests a multi-step transition, which could be an indication of a number of noncontiguous hydrophobic clusters. Thus each fragment contains one or more membrane-embedded and water-soluble subdomains. If that were so, the polypeptide backbone of the ATPase would have to weave in and out of the membrane a number of times. Investigation of additional proteolytic fragments will be needed to support this hypothesis. Preliminary experiments with Pronase indicated approximately 50–70% of the ATPase may be digested away leaving in the membrane a number of pep-

tides of low molecular weight. In any event, it is clear that the tryptic fragments are hydrophobic and are joined via a trypsin accessible link on the outside of the membrane bilayer thus requiring the ATPase polypeptide backbone to weave in and out of the membrane at least three times.

The ATPase is similar in this respect to the erythrocyte band 3 protein (Jenkins & Tanner, 1977) and to bacteriorhodopsin (Henderson & Unwin, 1975). The N and C termini of band 3 are located on one side of the membrane while a trypsin labile bond is located on the opposite side. Bacteriorhodopsin contains seven α helices which traverse the membrane bilayer joined by polypeptide sequences which are exposed to solution. It is not unreasonable to expect that a polypeptide chain which weaves in and out of the membrane is characteristic of membrane channel and pump proteins.

Fragment I_A has the ATP binding site and is extensively labelled with lactoperoxidase (Thorley-Lawson & Green, 1973; Stewart et al., 1976). Consistent with this evidence that a significant region of the fragment is exposed to solvent, fragment I_A is the least resistant to GdmCl denaturation. Fragment I_B which is largely protected from lactoperoxidase is the most resistant to GdmCl denaturation. It is interesting to note that from the amino acid compositions I_A rather than I_B should be the more hydrophobic peptide (Thorley-Lawson & Green, 1975).

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References

Auer, H. E., & Doty, P. (1966) *Biochemistry 5,* 1716.
Capaldi, R. A., & Vanderkooi, G. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69,* 930.
Chase, T., Jr., & Shaw, E. (1970) *Methods Enzymol. 3,* 20.
Corcoran, D., & Strittmatter, P. (1977) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 36,* 879.
Cortijo, M., Panijapan, B., & Gratzer, W. B. (1973) *Int. J. Peptide Protein Res. 5,* 179.
Dean, W. L., & Tanford, C. (1978) *Biochemistry 17,* 1683.
Degani, C., & Boyer, P. D. (1973) *J. Biol. Chem. 248,* 8222.
Edelhoch, H. (1967) *Biochemistry 6,* 1948.
Fish, W. W., Reynolds, J. A., & Tanford, C. (1970) *J. Biol. Chem. 245,* 5166.
Grefrath, S. P., & Reynolds, J. A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71,* 3913.
Hardwicke, P. M. D., & Green, N. M. (1974) *Eur. J. Biochem. 42,* 183.
Henderson, R., & Unwin, P. N. T. (1975) *Nature (London) 257,* 28.
Ikemoto, N., Sreter, F. A., & Gergely, J. (1971) *Arch. Biochem. Biophys. 147,* 571.
Inesi, G., & Scales, D. (1974) *Biochemistry 13,* 3298.
Jenkins, R. E., & Tanner, M. J. A. (1977) *Biochem. J. 161,* 139.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193,* 265.
MacLennan, D. H., Yip, C. C., Iles, G. H., & Seeman, P. (1972) *Cold Spring Harbor Symp. Quant. Biol. 37,* 469.
Meissner, G., Conner, G. E., & Fleischer, S. (1973) *Biochim. Biophys. Acta 298,* 246.
Nakashima, Y., & Konigsberg, W. (1974) *J. Mol. Biol. 88,* 598.
Nozaki, Y., Reynolds, J. A., & Tanford, C. (1978) *Biochemistry 17* (in press).
Racker, E., & Eytan, E. (1975) *J. Biol. Chem. 250,* 7533.
Rizzolo, L. J., le Maire, M., Reynolds, J. A., & Tanford, C. (1976) *Biochemistry 15,* 3433.
Steck, T. L., & Yu, J. (1973) *J. Supramol. Struct. 1,* 220.
Stewart, P. S., & MacLennan, D. H. (1974) *J. Biol. Chem. 249,* 985.
Stewart, P. S., MacLennan, D. H., & Shamoo, A. E. (1976) *J. Biol. Chem. 251,* 712.
Tajima, S., Enomoto, K., & Sato, R. (1976) *Arch. Biochem. Biophys. 172,* 90.
Tanford, C. (1968) *Adv. Protein Chem. 23,* 121.
Tanford, C., Pain, R. H., & Otchin, N. S. (1966) *J. Mol. Biol. 15,* 489.
Thorley-Lawson, D. A., & Green, N. M. (1973) *Eur. J. Biochem. 40,* 403.
Thorley-Lawson, D. A., & Green, N. M. (1975) *Eur. J. Biochem. 59,* 193.
Tomita, M., & Marchesi, V. T. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72,* 2964.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244,* 4406.
Wong, K. P., Roxby, R., & Tanford, C. (1971) *Anal. Biochem. 40,* 459.
Yamanaka, N., & Deamer, D. W. (1976) *Biochim. Biophys. Acta 426,* 132.