Molecular Weights and Hydrophobicity of the Polypeptide Chain of Sarcoplasmic Reticulum Calcium(II) Adenosine Triphosphatase and of Its Primary Tryptic Fragments†

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ABSTRACT: The polypeptide chain of the Ca^{2+}-stimulated adenosine triphosphatase from sarcoplasmic reticulum has a molecular weight of 119 000 ± 6500 on the basis of sedimentation equilibrium measurements in sodium dodecyl sulfate. The two primary fragments obtained by limited proteolysis each have within experimental error the same molecular weight, corresponding to one-half the molecular weight of the whole chain. Both fragments are equally resistant to complete denaturation by guanidine hydrochloride, a property characteristic of many intrinsic membrane proteins. This suggests that the native enzyme has two membrane-embedded halves, with an externally accessible link between them.

Estimates of the molecular weight of the polypeptide chain of the Ca^{2+}-ATPase[1] of sarcoplasmic reticulum, based on sodium dodecyl sulfate gel electrophoresis, have ranged from 90 000 to 115 000 (e.g., McFarland and Inesi, 1971; Louis and Shooter, 1972; Meissner et al., 1973; Warren et al., 1974). Similar estimates for the primary trypsin cleavage products range from 52 000 to 60 000 for the slower moving electrophoretic band (hereafter called fragment I) and from 45 000 to 55 000 for the faster moving electrophoretic band (hereafter called fragment II) (Thorley-Lawson and Green, 1973; Inesi and Scales, 1974; Louis et al., 1974; Stewart and MacLennan, (1974). We have observed a similar range of apparent molecular weights, depending on whether the method of Fairbanks et al. (1971) or of Weber and Osborn (1969) is used, and on whether or not 6 M urea is included. Molecular weights estimated by this method are subject to considerable uncertainty, since correct results can be expected only if the polypeptide under study interacts with sodium dodecyl sulfate in the same fashion as water-soluble proteins used as standards (Reynolds and Tanford, 1970b). Direct rather than empirical methods are required to obtain molecular weights of known reliability. Appropriate measurements for the intact (succinylated) polypeptide have been made by Hasselbach (1972), but agreement between different methods was poor, ranging from 94 000 (by osmotic pressure) to 130 000 (by sedimentation equilibrium). No direct measurements have been reported for the fragments.

This paper presents the results of molecular weight measurements by sedimentation equilibrium. Disulfide bonds must be reduced and a dissociating solvent must be used if polypeptide chain molecular weights are to be obtained, and we have employed sodium dodecyl sulfate for this purpose, as did Hasselbach in the measurements cited above. We also attempted to employ guanidine hydrochloride, but found that neither the intact chain nor the fragments could be dissociated to randomly coiled polypeptides in this solvent. This is, however, in itself an observation of considerable interest with implications for the overall structure of the ATPase.

Separation of the primary tryptic cleavage products from each other was a necessary step in this investigation. We confirmed the finding of Stewart (1975) that purification of the fragments can be achieved by hydroxylapatite chromatography in sodium dodecyl sulfate according to the procedure of Moss and Rosenblum (1972). It has since been shown that the fragments can also be separated by preparative gel electrophoresis (Thorley-Lawson and Green, 1975).

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. Guanidine hydrochloride was obtained from Heico Co. Iodoacetamide and iodoacetic acid (Sigma) were recrystallized from hexane. Sodium deoxycholate (Fisher) was purified by charcoal treatment and recrystallized from 80% acetone. ^{35}S-labeled sodium dodecyl sulfate was purchased from Amersham-Searle and [^{14}C]phosphatidylcholine was obtained from New England Nuclear Co. Trypsin, which has been purified by affinity chromatography on a chicken ovomucoid column, was a gift of Dr. Neal Robinson.

Purified ATPase vesicles were prepared by the method of Meissner et al. (1973). The samples were essentially identical to those described in a previous paper (le Maire et al., 1976) and contained about 78 mol of phospholipid/10^5 g of protein.

Preparation of ATPase Solutions. For measurements in $DodSO_4$, vesicles were dissolved in approximately 0.1 M $DodSO_4$ (weight ratio $DodSO_4$/protein > 2.5) and 0.1 M β-mercaptoethanol, and heated at 100 °C for 1–1.5 min. Free SH groups were alkylated by adding 1.5 mol of iodoacetamide/mol of β-mercaptoethanol, and incubating in the dark at pH 9.0 and 20 °C. The reaction was stopped after 30 min by lowering the pH to 6.0. Trace amounts of [^{14}C]phosphatidylcholine were added and the final solution was chromatographed on a Sepharose-4B column, preequilibrated and subsequently eluted with 3.4 mM $DodSO_4$, 10 mM phosphate buffer (pH 7), 1 mM NaN_3. The composition of this medium was designed to maximize $DodSO_4$ binding while remaining below the cmc of the detergent (Reynolds and Tanford, 1970a): both binding measurements and ultracentrifugation

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[1] Abbreviations used are: cmc, critical micelle concentration; ATPase, adenosine triphosphatase; BSA, bovine serum aluminum; CD, circular dichroism; $DodSO_4$, sodium dodecyl sulfate.

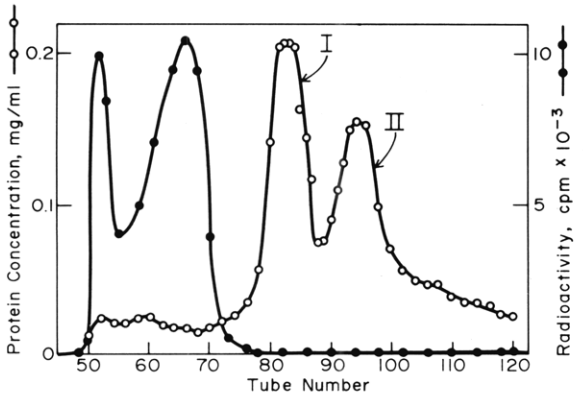

FIGURE 1: Sodium dodecyl sulfate–hydroxylapatite chromatography of the primary tryptic cleavage fragments. A linear gradient of 0.3–0.6 M phosphate buffer, pH 7.0, in 3.47 mM sodium dodecyl sulfate begins at tube 50 and ends at tube 180. Up to 40 mg of protein has been loaded on the column with similar resolution. Trace amounts of [^{14}C]phosphatidylcholine were added to mark the elution of phospholipid.

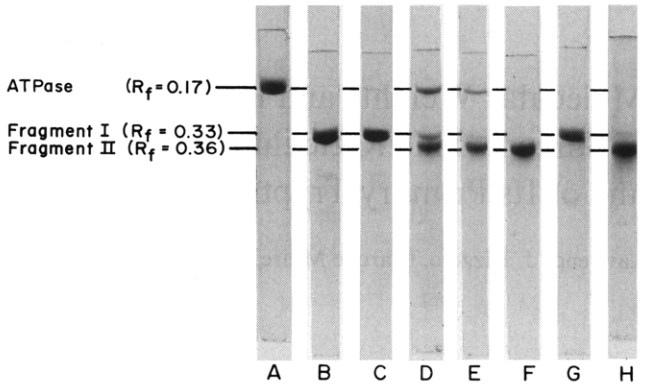

FIGURE 2: Sodium dodecyl sulfate gel electrophoresis. (A) ATPase, reduced and alkylated; (B–F) hydroxylapatite column: (B) tube 80, (C) tube 85, (D) tube 90, (E) tube 95, (F) tube 100; (G) fragment I, purified as shown in Figure 3; (H) fragment II, purified as shown in Figure 4. 15–20 μg of protein were loaded on each gel. The time of electrophoresis was not the same for all gels; bands of similar R_f (measured relative to the tracking dye) have been aligned.

experiments are facilitated if the presence of $DodSO_4$ micelles can be avoided. Fractions of the column eluate were analyzed for protein and $DodSO_4$ (see below) and the distribution of phospholipid was determined by following the radioactivity of the added tracer. Most of the lipid was found to be contained in $DodSO_4$–lipid micelles, but a small amount was incorporated in the protein–$DodSO_4$ complexes, at a level of about 0.05 g of lipid/g of protein.

For measurements in guanidine hydrochloride, vesicles were dissolved and chromatographed in deoxycholate as previously described (le Maire et al., 1976). No lipid is retained under these conditions. The eluted protein was diluted to bring the deoxycholate concentration below the cmc, and was then concentrated by ultrafiltration. Guanidine hydrochloride was added to a final concentration of 7 M, disulfide bonds were reduced and alkylated, and a final purification was achieved by chromatography on a Sepharose 4B column equilibrated with 6 or 7 M guanidine hydrochloride. (The sample used for circular dichroism measurements was a freshly reduced sample that had not been alkylated.)

Preparation of Primary Tryptic Fragments. Trypsin cleavage was carried out essentially as described by previous investigators (Ikemoto et al., 1971; Thorley-Lawson and Green, 1973; Inesi and Scales, 1974). 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 400:1 protein/trypsin weight ratio. The reaction was stopped by adding an excess of *p*-nitrophenyl *p*-guanidino benzoate. The product was dissolved in 0.1 M $DodSO_4$, 0.13 M β-mercaptoethanol, and heated for 2 min at 100 °C.

Crude separation of the fragments was achieved by chromatography on 0.9 × 30 cm hydroxylapatite columns, essentially as described by Moss and Rosenblum (1972). A linear gradient of 0.3–0.6 M phosphate buffer, pH 7.0, in 3.4 mM $DodSO_4$ (total volume 150 ml) was used for elution. This method was recommended to us by Dr. P. S. Stewart, who had previously used it for the same purpose (Stewart, 1975). We have observed that a fresh hydroxylapatite column has to be prepared for each use and that the resolution achieved varies from lot to lot. The crude fractions obtained by this method were dialyzed vs. 3.47 mM $DodSO_4$ in 0.01 M phosphate buffer (pH 7) and reduced and alkylated with iodoacetamide. The samples were concentrated by pressure filtration and chromatographed on Bio-Gel A 1.5 m, in the same $DodSO_4$-buffer medium. Fractions of the eluate were analyzed for protein and $DodSO_4$ (see below) and their purity with respect to polypeptide chain composition was examined by $DodSO_4$ gel electrophoresis.

For measurements in guanidine hydrochloride, $DodSO_4$ was removed by dialysis against 0.01 M phosphate buffer, which contained suspended mixed-bed ion-exchange resin to prevent buildup of the $DodSO_4$ concentration outside the dialysis bag. The fragments were then dialyzed against 6 M guanidine hydrochloride, and were reduced and alkylated a second time.

Protein Concentration. Column fractions were analyzed for protein 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 and Green (1974).

Sodium Dodecyl Sulfate Binding. Column fractions were analyzed for $DodSO_4$ by the methylene blue method of Ray et al. (1966), or by use of ^{35}S-labeled detergent (Reynolds and Simon, 1974). No radioactive lipid was present when the latter procedure was used. Binding of detergent to the protein was measured in terms of the excess $DodSO_4$ associated with the protein peak: essentially constant values across the peak were always obtained.

Sedimentation Equilibrium. Measurements were made with a Beckman Model E analytical ultracentrifuge equipped with photoelectric scanner, using appropriate fractions from the Sepharose 4B eluate. The results were corrected for bound detergent and lipid as described previously (Tanford et al., 1974). The slope of a semilogarithmic plot of the data formally yields the quantity $M_P(1 - \phi'\rho)$, where M_P is the molecular weight of the protein component of the sedimenting particle (excluding bound solvent, detergent or lipid), ϕ' is the effective specific volume per g of protein, and ρ is the solvent density. The parameter ϕ' can be eliminated by the relation

$$M_P(1 - \phi'\rho) = M_P[(1 - \bar{v}_P\rho) + \delta_D(1 - \bar{v}_D\rho) + \delta_L(1 - \bar{v}_L\rho)] \quad (1)$$

where δ_D and δ_L represent bound detergent and lipid, respectively, in g/g of protein, and $\bar{v}_P$, $\bar{v}_D$, and $\bar{v}_L$ represent the partial specific volumes of pure protein, detergent, and lipid. Values of $\bar{v}_P$ were calculated on the basis of amino acid compositions (Thorley-Lawson and Green, 1975) by the method of Cohn and Edsall (1943). We obtained $\bar{v}_P = 0.738$ cm^3/g for intact ATPase, identical to the measured value of Hasselbach (1972). Values of 0.737 and 0.736 cm^3/g were obtained for fragments

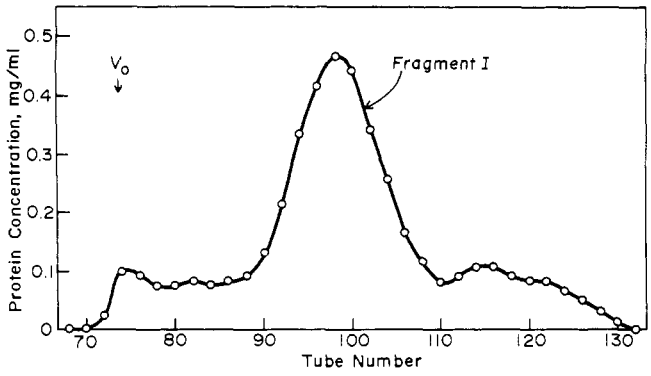

FIGURE 3: Gel filtration of fragment I. Pooled fractions from the hydroxylapatite column were reduced and alkylated with iodoacetamide loaded on a Bio-Gel A 1.5-m column (1.5 × 180 cm) and eluted with 0.02 M phosphate buffer, pH 7.0, in 3.47 mM sodium dodecyl sulfate. V_0 indicates the position of the void volume marker.

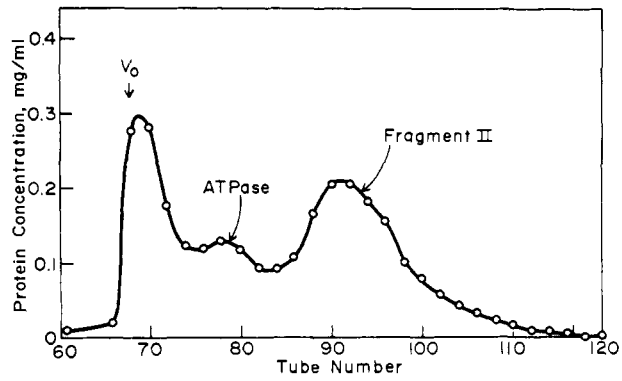

FIGURE 4: Gel filtration of fragment II: conditions as described in Figure 3. As shown by Figure 2 (D-F), fraction II from the hydroxylapatite column was more heterogeneous than fraction I. The purity of fragment II after gel filtration (tubes 86-95 were used) is indicated by Figure 2H.

I and II, respectively. The partial specific volumes for $DodSO_4$ (0.87 cm^3/g) and phospholipid (0.975 cm^3/g) were taken from Tanford et al. (1974), the latter being based on the lipid composition of ATPase vesicles given by MacLennan et al. (1971). The last term in eq 1 is in fact negligibly small, so that the precise value of $\bar{v}_L$ is not important.

Other Methods. $DodSO_4$ gel electrophoresis was performed by the method of Weber and Osborn (1969). As noted in the Introduction we have at times employed alternate techniques. Fragments I and II were not resolved when the method of Fairbanks et al. (1971) was used. Circular dichroism measurements were carried out using a Cary 60 spectropolarimeter with circular dichroism attachment.

Results

Purification of Fragments. The elution pattern from a hydroxylapatite column is shown in Figure 1. All phospholipid is seen to elute ahead of the fragments, in fractions containing only small amounts of protein. Fractions were examined by $DodSO_4$ gel electrophoresis with the results shown in Figure 2. The major component of the first principal protein peak is seen to be the slower moving of the two tryptic fragments (fragment I); the major component of the second peak is the faster moving tryptic fragment (fragment II). The fragments are seen not to be resolved from uncleaved ATPase and secondary trypsin cleavage products, and were further purified by gel chromatography, as described in the Experimental Section. Elution patterns are shown in Figures 3 and 4 and $DodSO_4$ gel electrophoretic patterns from fractions containing pure fragments are shown in Figure 2.

Sodium Dodecyl Sulfate Binding. As was noted in the Experimental Section, conditions used in these experiments were chosen to maximize $DodSO_4$ binding while remaining below the cmc of the detergent. Full saturation of the protein by the detergent was not achieved under these conditions. Sodium dodecyl sulfate binding to the intact chain was determined using ^{35}S-labeled sodium dodecyl sulfate to measure detergent concentration. Several measurements under the conditions of the ultracentrifuge experiments (3.47 mM free $DodSO_4$) yielded a binding level of 0.82 ± 0.08 g of $DodSO_4$/g of protein. At 2.81 mM free $DodSO_4$ the amount bound was 0.72 ± 0.03 g/g and at 4.20 mM free $DodSO_4$ it was 1.09 ± 0.05 g/g. A few determinations were made using the methylene blue method for measuring detergent concentration and somewhat higher apparent binding was observed. The discrepancy probably arises from the presence of phospholipid in the protein-detergent complexes, i.e., phospholipid may complex with methylene blue and be extracted into the organic phase along with $DodSO_4$. It is also possible that some protein may be extracted and contribute to the color yield. Since the results using ^{35}S-labeled sodium dodecyl sulfate are unambiguous, the discrepancy does not affect the final result for the ATPase chain.

Binding measurements for the fragments were made using the methylene blue method only. At 3.47 mM free $DodSO_4$ several measurements yielded 0.98 ± 0.15 g of $DodSO_4$/g of protein for fragment I and 1.07 ± 0.17 g of $DodSO_4$/g of protein for fragment II. Because no phospholipid is present in this case we are reasonably confident that the binding values obtained by the methylene blue method are correct. The higher binding observed (as compared to the intact chain at the same free $DodSO_4$ concentration) is not unreasonable because there is evidence for strong attraction between the fragments (Thorley-Lawson and Green, 1975), which may lead to the masking of hydrophobic surfaces in the intact chain. In addition, it is likely that the phospholipid bound in the ATPase-$DodSO_4$ complex replaces $DodSO_4$ that would be bound in the absence of lipid. The possible error in the binding measurement has in any event no significant effect on the molecular weight determination. If we were to assume that the fragments bind the same amount of $DodSO_4$ as the intact chain (0.82 g/g), the molecular weight values obtained would have been about 4000 higher than those reported in Table I, and their sum would have exceeded the nominal value for the molecular weight of the intact chain.

Binding to the intact ATPase at 3.47 mM free $DodSO_4$ was also measured at a higher ionic strength (33 mM phosphate instead of 10 mM), at which this detergent concentration is above rather than below the cmc. A binding ratio of about 1.3 g of $DodSO_4$/g of protein was obtained and retention of phospholipid in the ATPase-detergent complexes was no longer observed. This suggests that the internal cohesion between hydrophobic surfaces is disrupted above the cmc of the detergent. The result is of interest in view of the fact that the separation of fragments on hydroxylapatite is also carried out at 3.47 mM $DodSO_4$. The concentration of phosphate buffer in this procedure is 0.3-0.6 M.

Sedimentation Equilibrium. Ten or more measurements were made for each sample, over a range of initial protein concentrations and rotor speeds. Linear equilibrium plots were obtained under all conditions, except when the highest rotor speed and highest protein concentration were used simulta-

TABLE I: Summary of Results.[a]

	Range of Initial Protein concn (mg/ml)	Range of Rotor Speed (rpm)	$M_P(1 - \phi'\rho)$	$DodSO_4$ Binding (g/g of Protein)	Mol wt[c]
Whole ATPase	0.08–0.30	7 200–12 000	43 900 ± 2300	0.82 ± 0.05[b]	119 000 ± 6500
Fragment I	0.05–0.30	12 000–16 000	21 900 ± 1700	0.98 ± 0.15	56 000 ± 5000
Fragment II	0.06–0.30	12 000–16 000	23 900 ± 1300	1.07 ± 0.17	59 000 ± 4500

[a] Using eq 1. [b] Also contained phospholipid at 0.05 g/g of protein. [c] Standard deviation reflects combined error in $M_P(1 - \phi'\rho)$ and in the binding measurement.

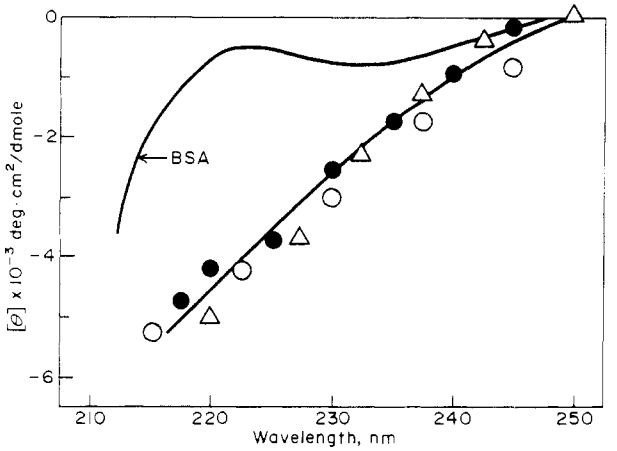

FIGURE 5: Circular dichroism spectra of reduced and alkylated proteins in 6 M guanidine hydrochloride. The points represent measurements for intact ATPase (●), fragment I (△), and fragment II (○): because low protein concentrations were used, differences between the samples are within experimental uncertainty. The curve for bovine serum albumin (BSA) is taken from Nozaki et al. (1974).

neously, under which conditions a slight decrease in slope near the bottom of the cell, reflecting thermodynamic nonideality, was observed. We conclude that each sample was homogeneous, with no tendency to aggregate at high protein concentration or to dissociate to species of lower molecular weight at low protein concentration. Results are summarized in Table I.

Experiments in Guanidine Hydrochloride. Sedimentation equilibrium experiments were performed in 6 M guanidine hydrochloride, after reduction of disulfide bonds by dithiothreitol and alkylation of SH groups by iodoacetamide. Curved plots were obtained, indicative of considerable aggregation. 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 the two fragments. Using iodoacetate as alkylating agent in place of iodoacetamide had no effect.

Circular dichroism spectra, shown in Figure 5, demonstrate that secondary structure is retained under these conditions. The spectra are clearly incompatible with the CD spectrum of a randomly coiled polypeptide, an example of the latter being included in the figure for comparison. The strong absorbance of guanidine hydrochloride precludes measurements below 215 nm, and the small differences between the results for intact ATPase and the fragments above that wavelength are within experimental error. If the results are compared with those obtained for polylysine in defined conformations (Greenfield and Fasman, 1969), we would judge about 60% of the ATPase polypeptide or its fragments to be randomly coiled, the remainder (presumably reflecting the chain segments where association occurs) being more likely predominantly in a β conformation than in an α-helical conformation.

When the reduced ATPase chain in 6 or 7 M guanidine hydrochloride is subjected to gel chromatography on Sepharose 4B, after calibration by the procedure of Fish et al. (1969),[2] an apparent "molecular weight" of 150 000 is obtained. This result obviously has no significance whatsoever for a polypeptide chain that has not assumed the random-coil conformation in this solvent.

Discussion

The molecular weight of the ATPase polypeptide chain determined in this investigation is at the upper limit of the broad range given by previous measurements (Hasselbach, 1972) or by the estimates from sodium dodecyl sulfate gel electrophoresis that were cited in the Introduction. The primary tryptic fragments have identical molecular weights within experimental error, and their sum accounts for the total molecular weight of the intact polypeptide. If more than one peptide bond is accessible to the action of trypsin, leading to deletion of a segment of polypeptide during proteolysis, the deleted segment would have to be very small. The same conclusion is reached from the good agreement between the sum of the amino acid compositions of the fragments and the overall composition of the ATPase (Thorley-Lawson and Green, 1975).

The reduced ATPase polypeptide chain and both fragments were found to be resistant to complete denaturation by guanidine hydrochloride: aggregation occurred in this normally dissociating solvent and optical measurements demonstrated retention of folded regions of the polypeptide backbone. This phenomenon is rare among water-soluble proteins (Tanford, 1968), and we have observed in this laboratory that complete unfolding and disaggregation also occurs for the apoproteins of serum lipoproteins (Smith et al., 1972; and unpublished data for HDL) and for the peripheral membrane protein, spectrin (Gwynne and Tanford, 1970). On the other hand, resistance to denaturation by guanidine hydrochloride may be a characteristic of most intrinsic membrane protein (Steck and Yu, 1973). We have observed such resistance in two membrane-associated proteins that have extended sequences of hydrophobic amino acid residues, namely the major erythrocyte glycoprotein (Segrest et al., 1972; Tomita and Marchesi, 1975) and the coat protein from a filamentous bacteriophage of the fd class (Nakashima and Konigsberg, 1974). Resistance to guanidine hydrochloride denaturation may prove to be diag-

[2] These experiments were carried out in collaboration with Dr. Jesper V. Moller.

nostic for hydrophobic sequences of this type: it is known that segments of polyleucine and polyphenylalanine that are incorporated into water-soluble polypeptides are not denatured by guanidine hydrochloride (Sage and Fasman, 1966; Auer and Doty, 1966).

It is of interest that *both fragments* of the ATPase behave as intrinsic membrane polypeptides by this criterion, suggesting that the native ATPase may have two membrane-embedded halves, with an external link, accessible to the action of trypsin, between them. The situation is in sharp contrast to that found for the well-characterized membrane protein cytochrome b_5, where tryptic cleavage leads to formation of two very different fragments, one highly polar and water-soluble and the other hydrophobic and containing the membrane-combining region of the native protein (Spatz and Strittmatter, 1971; Visser et al., 1975). Each ATPase fragment is of course much larger than the entire cytochrome b_5 molecule. Fragment I is known to be split into two smaller fragments when the action of trypsin is prolonged beyond the period required for the primary scission (Thorley-Lawson and Green, 1973; Inesi and Scales, 1974). It is likely that both fragments I and II will ultimately prove to contain polar and hydrophobic subdomains.

Added in Proof

Stewart et al. (1976) published a detailed report on tryptic fragments of the Ca^{2+} ATPase after the present paper was submitted for publication. By use of quite different (and partly more direct) procedures they also conclude that both fragments I and II include membrane-embedded portions of the ATPase polypeptide, and they provide additional data suggesting that fragment II is less accessible from the outer surface of the sarcoplasmic reticulum membrane than fragment I. They used a slightly different procedure to obtain pure primary fragments in that their molecular sieving step preceded hydroxylapatite chromatography, whereas we used the reverse order. Stewart et al. assigned nominal molecular weights of 55 000 and 45 000, respectively, to fragments I and II, basing these assignments on sodium dodecyl sulfate gel electrophoretic mobilities.

References

Auer, H. E., and Doty, P. (1966), *Biochemistry 5,* 1716.

Cohn, E. J., and Edsall, J. T. (1943), Proteins, Amino Acids and Peptides, New York, N.Y., Reinhold, p 372.

Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971), *Biochemistry 10,* 2606.

Fish, W. W., Mann, K. G., and Tanford, C. (1969), *J. Biol. Chem. 244,* 4989.

Greenfield, N., and Fasman, G. D. (1969), *Biochemistry 8,* 4108.

Gwynne, J. T., and Tanford, C. (1970), *J. Biol. Chem. 245,* 3269.

Hardwicke, P. M. D., and Green, N. M. (1974), *Eur. J. Biochem. 42,* 183.

Hasselbach, W. (1972), *Mol. Bioenerg. Macromol. Biochem. Meyerhof. Symp. 1970,* 149.

Ikemoto, N., Sreter, F. A., and Gergely, J. (1971), *Arch. Biochem. Biophys. 147,* 571.

Inesi, G., and Scales, D. (1974), *Biochemistry 13,* 3298.

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

Louis, C., Buonaffina, R., and Binks, B. (1974), *Arch. Biochem. Biophys. 161,* 83.

Louis, C., and Shooter, E. M. (1972), *Arch. Biochem. Biophys. 153,* 641.

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

MacLennan, D. H., Seeman, R., Iles, G. H., and Yip, C. C. (1971), *J. Biol. Chem. 246,* 2702.

McFarland, B. H., and Inesi, G. (1971), *Arch. Biochem. Biophys. 145,* 456.

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

Moss, B., and Rosenblum, E. N. (1972), *J. Biol. Chem. 247,* 5194.

Nakashima, Y., and Konigsberg, W. (1974), *J. Mol. Biol. 88,* 598.

Nozaki, Y., Reynolds, J. A., and Tanford, C. (1974), *J. Biol. Chem. 249,* 4452.

Ray, A., Reynolds, J. A., Polet H., and Steinhardt, J. (1966), *Biochemistry 5,* 2606.

Reynolds, J. A., and Simon, R. H. (1974), *J. Biol. Chem. 249,* 3937.

Reynolds, J. A., and Tanford, C. (1970a), *Proc. Natl. Acad. Sci. U.S.A. 66,* 1002.

Reynolds, J. A., and Tanford, C. (1970b), *J. Biol. Chem. 245,* 5161.

Sage H. J., and Fasman, G. D. (1966), *Biochemistry 5,* 286.

Segrest, J. P., Jackson, R. L., and Marchesi, V. T. (1972), *Biochem. Biophys. Res. Commun. 49,* 964.

Smith, R., Dawson, J. R., and Tanford, C. (1972), *J. Biol. Chem. 247,* 3376.

Spatz, L., and Strittmatter, P. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 1042.

Steck, T. L., and Yu, J. (1973), *J. Supramol. Struct. 1,* 220.

Stewart, P. S. (1975), *Fed. Proc., Fed. Am. Soc. Exp. Biol. 34,* 582.

Stewart, P. S., and MacLennan, D. H. (1974), *J. Biol. Chem. 249,* 985.

Stewart, P. S., MacLennan, D. H., and Shamoo, A. E. (1976), *J. Biol. Chem. 251,* 712.

Tanford, C. (1968), *Adv. Protein Chem. 23,* 121.

Tanford, C., Nozaki, Y., Reynolds, J. A., and Makino, S. (1974), *Biochemistry 13,* 2369.

Thorley-Lawson, D. A., and Green, N. M. (1973), *Eur. J. Biochem. 40,* 403.

Thorley-Lawson, D. A., and Green, N. M. (1975), *Eur. J. Biochem. 59,* 193.

Tomita, M., and Marchesi, V. T. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2964.

Visser, L., Robinson, N. C., and Tanford, C. (1975), *Biochemistry 14,* 1194.

Warren, G. B., Toon, P. A., Birdsall, N. J. M., Lee, A. G., and Metcalfe, J. C. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 622.

Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.