# Hydrodynamic Properties of the Chromaffin Granule Hydrogen Ion Pumping Adenosinetriphosphatase†

Gary E. Dean,‡,§ Pamlea J. Nelson,‡ William S. Agnew,‖ and Gary Rudnick*,‡
*Departments of Pharmacology and Physiology, Yale University School of Medicine, New Haven, Connecticut 06510*
*Received September 17, 1986; Revised Manuscript Received December 4, 1986*

ABSTRACT: We have determined the hydrodynamic properties of detergent-solubilized ATPase, which is coupled to $H^+$ pumping in bovine adrenal chromaffin granules, by sedimentation equilibrium centrifugation and gel permeation chromatography. The protein solubilized with detergent containing phosphatidylserine sediments as a particle of 264 000 daltons and partial specific volume 0.829 $cm^3/g$. Assuming a protein $\bar{v}$ of 0.73 and using the $\bar{v}$ measured for detergent and lipid mixed micelles of 0.93 $cm^3/g$, we calculated that the protein component has a mass of 134 000 daltons and that the equivalent of approximately 1.5 micelles of detergent are bound per particle. The particle exhibits a Stokes radius of 43 Å, which, together with the calculated particle volume, indicates an axial ratio close to 1. We conclude that the ATPase is an intrinsic membrane protein with a structure very different from that of mitochondrial $F_1F_o$ ATPase.

ATP-driven $H^+$ pumps are responsible for acidifying a variety of intracellular organelles belonging to the vacuolar system, including lysosomes, endosomes, coated vesicles, and most secretory granules (Rudnick, 1986a,b; Mellman et al., 1986). Evidence from many laboratories suggests that these enzymes constitute a new class of ATP-driven $H^+$ pumps distinct both from the $E_1E_2$ types found in the plasma membranes of gastric parietal cells, yeast, fungi, and higher plants and also from the $F_1F_o$ ATPases of mitochondria, chloroplasts, and bacteria, which normally function as ATP synthetases. These two previously described types of $H^+$ pump differ in their catalytic properties. The $E_1E_2$ enzymes are strongly inhibited by vanadate and proceed through a phosphorylated intermediate (Goffeau & Slayman, 1982), while the $F_1F_o$ ATPases are inhibited by azide and are apparently not phosphorylated as part of the catalytic cycle (Linnett & Beechey, 1979; Boyer et al., 1982). The $F_1F_o$ ATPase is a multisubunit complex consisting of a membrane-embedded $F_o$ portion mediating transmembrane $H^+$ movement and an associated $F_1$ ATPase complex (Tzagoloff et al., 1973). Both $F_o$ and $F_1$ components are composed of smaller subunits. $F_1$ in particular is a 380 000-dalton (Da) complex containing at least four different subunits. The masses of $E_1E_2$ ATPases, in contrast, are approximately 100 000 Da (Kyte, 1981; Goffeau & Slayman, 1982).

The vacuolar ATPase is distinct in its inhibitor sensitivity, being insensitive to azide and vanadate but very sensitive to sulfhydryl reagents such as *N*-ethylmaleimide (NEM) (Dean et al., 1984, 1986). Little is known about its mechanism or structure. Efforts to isolate a phosphoenzyme intermediate have not succeeded (Forgac & Cantley, 1984), and some workers have proposed that the vacuolar ATPase is closely related to $F_1F_o$ (Apps & Schatz, 1979). Cidon and Nelson (1983) demonstrated that extraction with 2 M NaBr, which denatures and removes $F_1$ from mitochondrial membranes, does not affect 70% of the ATPase activity in purified chromaffin granule membranes. They also showed that antibodies against the $\beta$-subunit of mitochondrial $F_1$ ATPase do not react with the NaBr-stripped chromaffin granule membranes. Subsequently, Cidon et al. (1983) showed that the stripped membranes could be reconstituted with phospholipids and detergent to form vesicles in which ATP drives $H^+$ transport. These data argue strongly that mitochondrial $F_1F_o$ ATPase was not the $H^+$ pump of chromaffin granules.

Their data do not exclude the possibility that the chromaffin granule ATPase has a structure similar to $F_1F_o$, with a large, multisubunit ATP hydrolase bound to a hydrophobic $H^+$-conducting complex. In this paper, we show that the hydrolytic activity resides on a protein which is much smaller than $F_1$ and which behaves like an integral membrane protein. These properties are inconsistent with a structure like that of $F_1F_o$ ATPase.

## Experimental Procedures

*ATPase Preparation.* Chromaffin granule membranes were prepared as described by Cidon and Nelson (1983). The membranes were suspended on ice at a concentration of 10 mg $mL^{-1}$ in 10 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS), pH 7.5, containing 0.3 M sucrose, 5 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM ATP. A solution of 10% (w/v) octyl glucoside and 5% (w/v) sodium cholate, pH 7.5, was added, with mixing, to a final concentration of 1% and 0.5%, respectively, and the suspension was incubated on ice for 10 min. Insoluble material was removed by centrifugation at 39000*g* at 4 °C for 20 min, and dithiothreitol was added to a final concentration of 5 mM. ATPase activity was precipitated from solution by dropwise addition of an equal volume of a saturated ammonium sulfate solution followed by stirring for 1 h on ice. The precipitate was collected by centrifugation at 10000*g* at 4 °C for 10 min and resuspended in a minimal volume of 20 mM NaCl containing 40 mM tris(hydroxymethyl)aminomethane (Tris) base adjusted to pH 7 with 2-(*N*-morpholino)ethanesulfonic acid (MES), 0.02% (w/v) azide, 1% (w/v) polyoxyethylene(9) lauryl ether ($C_{12}E_9$), 4 mM ATP, 4 mM dithiothreitol, and 0.4 mg $mL^{-1}$ egg yolk phosphatidylserine (Airfuge buffer). Residual ammonium sulfate, octyl glucoside, and cholate were removed by centrifugation of 80-$\mu$L portions through 1-mL columns of Sephadex G-50 equilibrated in the same buffer.

† This work was supported by U.S. Public Health Service Grant HL-21217 to G.R.
‡ Department of Pharmacology.
§ Present address: Department of Microbiology and Molecular Genetics, University of Cincinnati College of Medicine, Cincinnati, OH 45267.
‖ Department of Physiology.

0006-2960/87/0426-2301$01.50/0 

The eluate, containing approximately 1.7 mg of protein, was stored on ice for a maximum of 24 h before use.

The ATPase activity in this preparation is almost exclusively due to the chromaffin granule $H^+$ pump by the following criteria: (1) Most of the ATPase activity in the membrane preparation comprising the starting material is coupled to $H^+$ pumping (Dean et al., 1986). (2) The ammonium sulfate precipitate, if resuspended in cholate or octyl glucoside–cholate mixtures, will reconstitute ATP-driven $H^+$ pumping in liposomes. (3) The ATPase activity of the $C_{12}E_9$-solubilized preparation is at least 70% inactivated by removal of dithiothreitol and treatment with *N*-ethylmaleimide. Contaminating ATPase activity can be completely accounted for by Na,K-ATPase and mitochondrial $F_1F_o$ ATPase (Dean et al., 1986). To correct for the presence of these enzymes, all assays were performed in the presence of ouabain and efrapeptin. We have established that ATPase activity measured under these conditions represents the enzyme which, in the intact membrane, is coupled to $H^+$ pumping and drives amine transport (Dean et al., 1986).

*Equilibrium Sedimentation.* Sedimentation experiments were carried out with the Beckman Airfuge, a desk-top air turbine microcentrifuge, according to procedures modified from those first reported by Bothwell et al. (1978). This approach requires only that one be able to measure small amounts of the protein to be characterized and that the protein be monodisperse in solution. The upper limit of protein molecular weight which can be analyzed by this method has been extended from the value of approximately $10^5$ first indicated by Bothwell et al. (1978) to more than $10^6$ by partially floating the proteins in solutions of higher density, containing either $D_2O$ or $D_2{}^{18}O$. Beyond this, as employed here, systematic variation in solvent density can be used to ascertain the partial specific volume of an unknown protein, according to the rationale first described in analytical ultracentrifugation studies by Edelstein and Schachman (1967).

Samples of either standard proteins or the chromaffin granule ATPase (approximately 5 mg of protein/mL) were prepared in Airfuge buffer containing 2.5 mg/mL T-40 dextran. Aliquots of 120 μL were delivered to each of the six 220-μL cellulose propionate tubes of the A-100/30 rotor (Beckman part 341288). The Airfuge was operated at approximately 4 °C at a setting of 4 psi, or about 25 000 rpm. Precise rotational velocities were measured in each experiment with a General Radio Co. Strobotac Type 1531-A beginning 0.5 h after the start of the run. Several determinations were made throughout each experiment. Rotational velocities varied less than 4% during the course of these experiments, and the time-average velocity was used in these calculations. After 22–24 h, the rotor was decelerated without braking to minimize mixing. Each tube was withdrawn from the rotor and the upper 40 μL carefully removed for assay. The bottom 80 μL was then thoroughly mixed and 40 μL removed for assay. The chromaffin granule ATPase was assayed in the presence of 2 μM efrapeptin and 100 μM ouabain using $^{32}P$ release from [$\gamma$-$^{32}$P]ATP as described previously (Dean et al., 1986). The fractional concentration ($F$) was computed as the ratio of the concentration in the upper 40 μL to the total average concentration in the 120-μL sample.

Control experiments indicated that with the A-100/30 rotor at least 20 h was required for complete equilibrium to be attained as compared to approximately 8 h with the A-100/18 rotor (W. S. Agnew and H. F. Rudnick, unpublished results). Failure to include minimal concentrations of dextran in the samples resulted in inconsistent data, with scattering toward lower apparent molecular weights, clearly the result of convectional mixing. In addition, in the course of experiments measuring sedimentation at different solution densities, it became evident that even though identical samples were carefully balanced against one another in the rotor, if the range of densities in the three sets of duplicate samples was greater than 0.0337 g/cm$^3$ the resulting data were often scattered, apparently the result of mixing caused by rotor precession during deceleration.

The standard curve (illustrated in Figure 1) was constructed from results obtained from more than 18 experiments with soluble proteins of known molecular weight, and partial specific volume. These proteins, including bovine serum albumin, ovalbumin, yeast alcohol dehydrogenase, lactic acid dehydrogenase, catalase, horseradish peroxidase, aldolase, and glyceraldehyde dehydrogenase, were sedimented at varying rotational velocities or at varying solution densities. All of the results yielded a consistent relationship between log $F$ and the reduced molecular weight, $\sigma$, defined as

$$\sigma = [M_r(1 - \bar{v}\rho)\omega^2]/2RT \quad (1)$$

where $M_r$ is the molecular weight, $\bar{v}$ is the partial specific volume, $\rho$ is the solution density, $\omega$ is the angular velocity, $T$ is the absolute temperature, and $R$ is the gas constant. Thus, $\sigma$ could be controlled by varying the speed of centrifugation, the solution density, or the molecular weight of the standard. It is in principle possible to calculate the theoretical relationship between log $F$ and $\sigma$. However, the value of $F$ depends not only on the physical parameters of the protein and solution ($M_r$, $\bar{v}$, and $\rho$) and the rotational velocity ($\omega$) but also on the geometry of the column of liquid during centrifugation, and upon the tendency of the solution to reorient when the tube is removed from the rotor and placed vertically before collecting the fractions for assay. Because of this, the standard curve was constructed. The data were fitted to a linear regression of log $F = 0.1765\sigma - 0.2009$. For each unknown, $F$ was determined, and $\sigma$ was calculated from this relation.

*Gel Filtration.* Samples of standards and chromaffin granule ATPase prepared as described above were applied to a 7.5 × 600 mm LKB TSK 4000 column equilibrated in 10 mM Tris–MES, pH 7.0, containing 0.1% $C_{12}E_9$ and eluted with the same buffer at a rate of 0.5 mL min$^{-1}$. The void volume was determined with blue dextran and the total column volume with $^3H_2O$. Elution positions of the standards and blue dextran were determined by the absorbance at 280 nm, by liquid scintillation counting in the case of $^3H_2O$, and by ATPase assay in the case of chromaffin granule ATPase. *N*-Ethylmaleimide-sensitive ATPase activity was determined by subtracting from the total ATPase activity that which was measured after the sample was treated for 5 min with 1 mM *N*-ethylmaleimide.

## Results

*Equilibrium Sedimentation of Chromaffin Granule ATPase.* As described by Bothwell et al. (1978), the log of the fractional concentration of a protein remaining in the upper portion of the centrifuge tube is a monotonic function of the reduced molecular weight ($\sigma$ in eq 1). This relationship is illustrated in Figure 1 constructed from the sedimentation behavior of a series of reference proteins. To determine the molecular weight of a soluble protein, of known partial specific volume, log $F$ is determined experimentally, and $\sigma$ is estimated from the standard curve. From knowledge of the solvent density ($\rho$) and the rotational velocity, $M_r$ may be calculated from eq 1.

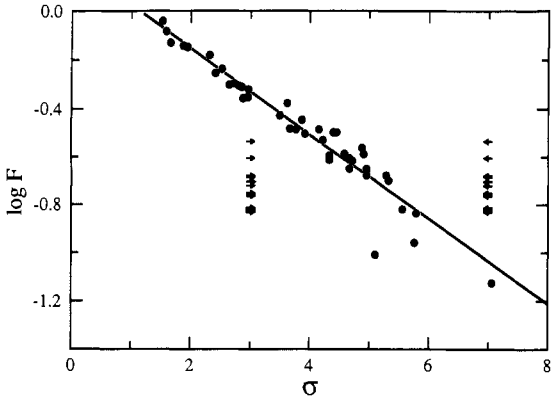


FIGURE 1: log of fractional concentration vs. reduced molecular weight. Standard proteins, including bovine serum albumin, ovalbumin, yeast alcohol dehydrogenase, catalase, β-galactosidase, and glyceraldehyde-3-phosphate dehydrogenase, were sedimented under different conditions of solvent density or rotational velocity. The log $F$, computed as described under Experimental Procedures, was related to the computed value for $\sigma$. On this curve, no attempt has been made to distinguish points derived from different proteins, or from the same protein run under different centrifugal or solvent conditions. All data fit the indicated relationship equally well, regardless of the manner in which they were generated (W. S. Agnew, H. F. Rudnick, and M. C. Emerick, unpublished results). The data were fit to a linear regression of $\log F = 0.1765\sigma - 0.2009$. From this relation, values of $\sigma$ were computed for the chromaffin granule ATPase from experimentally measured values of log $F$. The arrows indicate values of log $F$ measured for the ATPase, showing they fell in a range in which the method is reliable.

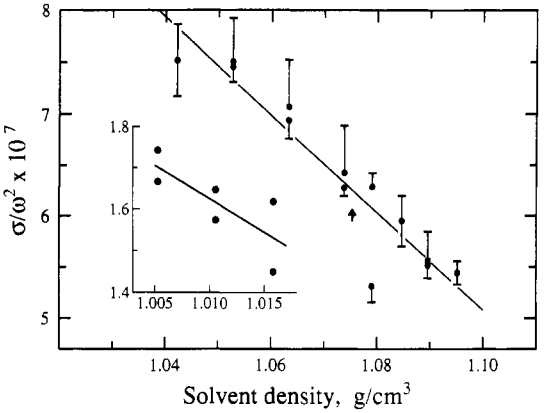


FIGURE 2: Buoyancy of chromaffin granule ATPase vs. solvent density. The fractional distribution ($F$) of ATPase activity after sedimentation to equilibrium was determined as described under Experimental Procedures. $\sigma/\omega^2$ values were calculated from measured values of $F$ by using the standard curve in Figure 1. Each point is the result of at least three determinations. The error bars show the standard error of the mean of the experimental points. The arrow indicates the solvent density equal to $1/\bar{v}$ for the detergent–lipid mixed micelle in the absence of protein. *Inset*: Buoyancy of $C_{12}E_9$–phosphatidylserine mixed micelles vs. solvent density. Airfuge buffer containing tracer amounts of [$^{14}$C]phosphatidylcholine was prepared as described under Experimental Procedures, and the detergent–lipid mixed micelles were sedimented as described for solubilized chromaffin granule ATPase in medium containing 5%, 10%, and 15% $D_2O$. $\sigma/\omega^2$ was calculated from measured values of $F$ as described in the text.

For detergent-solubilized proteins, the determination is complicated because the sedimenting particle includes bound detergent and, in many instances, lipid. Estimating the molecular weight of such a protein requires knowledge of the relative proportions of protein, lipid, and detergent, and the $\bar{v}$ of the lipid and detergent components. This information may be obtained from measurements of $F$ in solvents of various densities. For each determination, $\sigma/\omega^2$ is plotted as a function of solvent density. Equation 1 predicts that $\sigma/\omega^2$ should decrease linearly with solvent density, and the parameters of a linear fit to the data yield $\bar{v}$ for the protein–detergent–lipid complex and the molecular weight of the total particle, $M_r(o)$. The relationship between the buoyant mass of the particle and the molecular weight of the protein $M_r(p)$ is described by eq 2, where $\bar{v}(p)$ and $\bar{v}(d)$ are the partial specific volumes of the

$$M_r(o)(1 - \bar{v}\rho) = M_r(p)\{[1 - \bar{v}(p)\rho] + \delta[1 - \bar{v}(d)\rho]\} \quad (2)$$

protein and of the bound detergent and lipid, respectively, and $\delta$ is the mass ratio of bound detergent to protein.

Thus, $\sigma/\omega^2$ measured at a solvent density equal to the reciprocal of the partial specific volume of the bound detergent yields the buoyant mass of the protein portion alone [e.g., the $\delta[1 - \bar{v}(d)\rho]$ term in eq 2 becomes a zero]. Making reasonable assumptions as to the likely value of $\bar{v}(p)$, the mass of the protein may be derived, together with $\delta$. Alternatively, the value of $\delta$ may be obtained from the relationship

$$\delta = [\bar{v}(o) - \bar{v}(p)]/[\bar{v}(d) - \bar{v}(o)] \quad (3)$$

where $\bar{v}(o)$ is the partial specific volume of the particle. In this case, $M_r(p)$ may be derived from each estimate of $M_r(o)$ at each solvent density tested, by dividing by $(1 + \delta)$. The mean and standard deviation may be estimated from the tabulated data.

The arrows in Figure 1 point to the values of $F$ measured for the chromaffin granule ATPase solubilized in $C_{12}E_9$ in the presence of phosphatidylserine, over a range of solution densities obtained by substituting various amounts of water with $D_2O$. The values of $F$ measured in this way fall clearly within the range covered by the standard curve.

For each value of log $F$, $\sigma$ was calculated from the linear regression fit of the standard curve. As illustrated in Figure 2, $\sigma/\omega^2$ was then plotted against the solution density, $\rho$. In these experiments, increasing $\rho$ from 1.042 g/cm$^3$ (40% $D_2O$) to 1.095 g/cm$^3$ (90.32% $D_2O$) caused $\sigma/\omega^2$ to decrease from $7.70 \times 10^{-7}$ to $5.44 \times 10^{-7}$ s$^2$/cm$^2$. To within experimental error, the data describe the expected linear relationship between density and buoyant mass. Linear least-squares analysis of these data yields a slope of $(-4.75 \pm 0.35) \times 10^{-6}$ s$^2$·cm/g and an intercept of $(5.73 \pm 0.37) \times 10^{-6}$ s$^2$/cm$^2$. Using eq 1, we obtain values of $0.83 \pm 0.01$ cm$^3$/g for $\bar{v}(o)$ and 264 000 ± 17 000 Da for the total particle mass.

To determine the fraction of the particle represented by proteins, it was necessary to determine the partial specific volume of the lipid–detergent portion of the particle. Assuming the density of bound lipid and detergent is not significantly different from that in micellar form, we sedimented to equilibrium a suspension of lipid–detergent mixed micelles containing trace amounts of 1,2-di[1-$^{14}$C]palmitoyl-L-3-phosphatidylcholine (Amersham, 120 mCi/mmol) in solutions containing 5%, 10%, and 15% $D_2O$. The micellar distribution, $F$, was computed from the distribution of radioactivity. The resulting values of $\sigma/\omega^2$ plotted against solution density (Figure 2, inset) yielded a partial specific volume of $0.93 \pm 0.02$ cm$^3$/g, and the computed mass of the micelles was 83 000 ± 31 000 Da.

At a solvent density equal to the detergent–lipid micelle density, the bouyant mass of the ATPase is only that of the protein constituent. This density is indicated in Figure 2 by the arrow. Assuming that the partial specific volume of the protein is 0.73, and using the interpolated value of $\sigma/\omega^2$, the calculated protein mass is 134 000 ± 22 000 Da [the uncertainty in this value derives mainly from the contribution of the uncertainty in $\bar{v}(d)$ but does not include the uncertainty associated with the estimated value of $\bar{v}(p)$ (see Discussion)]. The particle is thus composed of almost equal amounts of

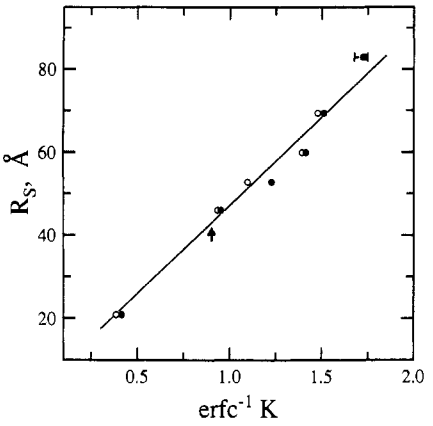


FIGURE 3: Stokes radius for standard proteins measured in the presence (●) and absence (○) of detergent is plotted against the erfc$^{-1}$ $K$ according to the rationale of Ackers (1967) where $K$ is calculated as $(V_e - V_0)/(V_t - V_0)$ as described under Experimental Procedures. Standards were thyroglobulin (82.9 Å), β-galactosidase (69.4 Å), apoferritin (59.9 Å), catalase (52.8 Å), yeast alcohol dehydrogenase (46 Å), and carbonic anhydrase (20.9 Å). The arrow indicates the elution position of chromaffin granule ATPase.

protein and detergent ($\delta = 0.97$). Assuming that the distribution of detergent and lipid in bound form is the same as that in the mixed micelles overall, the particle consists of 51% protein, 47% detergent, and 2% lipid.

*Gel Filtration of the Chromaffin Granule ATPase.* As an independent measure of the size of the detergent-solubilized ATPase, we performed high-performance liquid chromatography (HPLC) gel permeation chromatography. Under the conditions used, NEM-sensitive ATPase activity eluted as a single peak, indicating that it was present in the extracts in monodisperse form.

A series of standard proteins, of known Stokes radius, were eluted through the column, either in the presence or in the absence of 0.1% $C_{12}E_9$. The partition coefficient for each protein, computed as $K = (V_e - V_0)/(V_t - V_0)$, was plotted according to the convention of Ackers (1967), as illustrated in Figure 3. It is apparent that the detergent had little effect on the elution behavior of these proteins, indicating the sieving properties of the column are not modified. The arrow in this figure indicates the elution position of the chromaffin granule ATPase, defined as the peak of NEM-sensitive ATP hydrolysis. The Stokes radius derived from these studies was 43 ± 4 Å. This value is entirely consistent with the results of the sedimentation equilibrium measurements.

## Discussion

From the combined results of the sedimentation equilibrium studies and the gel permeation chromatography, all of the major physical parameters of the $C_{12}E_9$-solubilized chromaffin granule ATPase may be deduced. These are presented in Table I. It is noteworthy that such data are obtained even in a mixture with other proteins. Because we used an activity assay specific for the chromaffin granule ATPase, the preparation's lack of purity does not compromise the results obtained by this method. We have established that ATPase activity measured under these conditions represents the enzyme which, in the intact membrane, is coupled to $H^+$ pumping and drives amine transport (Dean et al., 1986).

The molecular weight of the detergent-solubilized ATPase is 264 000, and the partial specific volume of the particle is 0.829 cm$^3$/g. The partial specific volume of the detergent–lipid mixed micelles, 0.93 cm$^3$/g, combined with the conventional assumption for the partial specific volume of the protein element of 0.73 cm$^3$/g (*CRC Handbook*, 1970), yields the protein

Table I: Hydrodynamic Properties of the Chromaffin Granule ATPase[a]

| property | value |
|---|---|
| particle partial specific volume, $\bar{v}$(o) (cm$^3$/g) | 0.83 ± 0.01 |
| particle mass, $M_r$(o) (g/mol) | 264 000 ± 17 000 |
| detergent partial specific volume, $\bar{v}$(d) (cm$^3$/g) | 0.93 ● 0.02 |
| lipid–detergent mixed micelle mass, $M_r$(d) (g/mol) | 83 000 ± 30 000 |
| protein mass in particle, $M_r$(p) (g/mol) | 134 000 ± 22 000 |
| detergent–lipid mass in particle, $M_r$(o) − $M_r$(p) (g/mol) | 130 000 ± 20 000 |
| detergent–lipid/protein mass ratio, $\delta$ | 0.97 |
| Stokes radius, $R_s$ (Å) | 43 ± 4 |
| frictional coefficient, $f/f_0$ | 0.98 |
| axial ratio, $a/b$ | 1.0 |
| sedimentation coefficient, $s_{20,w}$ (S) | 9.2 |
| diffusion coefficient, $D_{20,w}$ (F) | 5.0 |

[a] Measurements and calculations are described in the text; the value of $\bar{v}$(p) = 0.73 cm$^3$/g has been assumed.

molecular weight of 134 000. Since the molecular weight calculations involve the term $1 - \bar{v}$, and most proteins have a $\bar{v}$ from 0.70 to 0.75, an error of 1% in $\bar{v}$ leads to roughly a 3% error in the molecular weight. Approximately 0.93 g of detergent and lipid is estimated to be bound per gram of protein, the equivalent of roughly 1.5 micelles. This value is consistent with the mass of the particle evaluated by extrapolating the line in Figure 2 to the density 1.37 g/cm$^3$, the reciprocal of $\bar{v}$(p). The Stokes radius, measured by gel filtration, was 43 Å. The frictional coefficient calculated from eq 4 is 0.98. This close agreement between the measured

$$f/f_0 = \frac{R_s}{[3M_r(o)\bar{v}(o)/4\pi N]^{1/3}} \quad (4)$$

hydrodynamic radius and that predicted from the measured partial specific volume and overall molecular weight indicates that the particle is quite spherical, with an axial ratio indistinguishable from 1.0 (Tanford, 1961), and also strengthens our confidence in the measured values. As indicated in the legend to Table I, from the molecular weight, partial specific volume, and the Stokes radius of the particle, the sedimentation coefficient ($s_{20,w}$) and diffusion coefficient ($D_{20,w}$) were computed to be 9.2 S and 5.0 F, respectively.

The large amount of detergent and lipid associated with the enzyme strongly suggests that it is closely associated with the lipid bilayer by hydrophobic interactions. This contrasts with the $F_1F_o$ ATPases whose hydrolytic $F_1$ components are relatively water-soluble proteins which associate with the membrane-bound $F_o$ component by protein–protein interactions (Tzagoloff et al., 1973). The chromaffin granule ATPase is also much smaller than the $F_1F_o$ ATPase, with the hydrolytic $F_1$ portion alone a multisubunit complex of 340 000–400 000 Da. In contrast, our best estimate of the chromaffin granule ATPase's molecular mass, 134 000 Da, is closer to the molecular masses of various $E_1E_2$ ATPases, which range from 96 000 to 112 000 Da (Goffeau & Slayman, 1982; MacLennan et al., 1985; Schull et al., 1985). The $E_1E_2$ enzymes are also intrinsic membrane proteins, like the chromaffin granule ATPase. Despite these apparent structural similarities, the chromaffin granule ATPase cannot be classified as an $E_1E_2$ enzyme since, like all other vacuolar ATPases studied, it is vanadate insensitive (Rudnick, 1986a,b).

The samples of $C_{12}E_9$-solubilized chromaffin granule ATPase which have been sedimented in the Airfuge for 24 h have not been shown to be active in reconstituting $H^+$ pumping in liposomes. This consideration raises the possibility that the 134 000-Da ATPase may not represent the entire ATP-driven

$H^+$ pump and that components which are required for $H^+$ translocation dissociate from the ATPase during the procedure. Given an axial ratio of 1.0, it is likely that the enzyme is deeply embedded in the lipid–detergent micelle, as expected for a transmembrane protein. Furthermore, the ATPase mass is sufficient, by analogy to the $E_1E_2$ ATPases, to account for an ATP-driven ion pump. It is premature, however, to conclude that the $C_{12}E_9$-solubilized ATPase represents the holoenzyme.

Cidon and Nelson (1986) have purified the chromaffin granule ATPase to a specific activity of 17.6 $\mu$mol $mg^{-1}$ $min^{-1}$. Their preparation yielded, on polyacrylamide gel electrophoresis, polypeptide bands of apparent $M_r$ 115000, 72000, 57000, and 39000. Percy et al. (1985) described a purified preparation of the chromaffin granule ATPase of much lower specific activity (0.47 $\mu$mol $mg^{-1}$ $min^{-1}$) which contained polypeptides of apparent $M_r$ 70000, 41000, 33000, and 16000 and a band at $M_r$ 140000 which was described as an aggregation artifact. We have reproduced Cidon and Nelson's preparation with similar results (specific activity 2.4 $\mu$mol $mg^{-1}$ $min^{-1}$) (data not shown). Given the experimental uncertainty associated with our measurements, a mass of 134 kDa for the holoenzyme could account for one copy each of either the 72-, 57-, and 39-kDa peptides or the 70-, 41-, 33-, and 16-kDa peptides.

Assuming a specific activity of 18 $\mu$mol $mg^{-1}$ $min^{-1}$ for homogeneous enzyme (Cidon & Nelson, 1986), a molecular mass of 134 kDa would indicate a turnover number of approximately 40 $s^{-1}$. Alternatively, if the turnover number is as high as 700 $s^{-1}$, then a molecular weight of $2.3 \times 10^6$ would be required to account for the observed specific activity. These turnover numbers are not unreasonable when compared to those measured for other ATP-driven ion pumps (Jorgensen, 1975; Penefsky, 1985).

Estimations of the molecular mass of various vacuolar ATPases by radiation inactivation (Bowman et al., 1986) and sedimentation velocity (Cidon & Nelson, 1983; Xie & Stone, 1986; Forgac & Berne, 1986) have yielded values of 200–530 kDa. Whether the larger masses represent multimers of the 134-kDa ATPase reported here or contain additional polypeptides not associated with the 134-kDa ATPase is impossible to determine from the present data. However, in the case of the analogous coated vesicle ATPase, Xie and Stone (1986) reported a molecular mass of 530 kDa while Forgac and Berne (1986) reported a mass of 200–250 kDa for the same enzyme. The difference is probably related to the fact that the preparation used by Xie and Stone was reconstitutively active while Forgac and Berne's preparation required addition of a particulate fraction for reconstituting ATP-driven $H^+$ pumping in proteoliposomes. If similar considerations apply with the chromaffin granule ATPase, it would suggest that the 134-kDa ATPase measured here represents the ATP-hydrolyzing component of a larger $H^+$ pump.

**Registry No.** ATPase, 9000-83-3.

## REFERENCES


Ackers, G. (1967) *J. Biol. Chem. 242*, 3237–3238.

Apps, D. K., & Schatz, G. (1979) *Eur. J. Biochem. 100*, 411–419.

Bothwell, M. A., Howlett, G. J., & Schachman, H. K. (1978) *J. Biol. Chem. 253*, 2073–2077.

Boyer, P. D., Kohlbrenner, W. E., McIntosh, D. B., Smith, L. T., & O'Neal, C. C. (1982) *Ann. N.Y. Acad. Sci. 402*, 65–83.

Cidon, S., & Nelson, N. (1983) *J. Biol. Chem. 258*, 2892–2898.

Cidon, S., & Nelson, N. (1986) *J. Biol. Chem. 261*, 9222–9227.

*CRC Handbook of Biochemistry and Molecular Biology* (1970) (Sober, M. A., Ed.) 2nd ed., pp C10–C12, Chemical Rubber Publishing Co., Cleveland, OH.

Dean, G. E., Fishkes, H., Nelson, P., & Rudnick, G. (1984) *J. Biol. Chem. 259*, 9569–9574.

Dean, G. E., Nelson, P. J., & Rudnick, G. (1986) *Biochemistry 25*, 4918–4925.

Edelstein, S. J., & Schachman, H. K. (1967) *J. Biol. Chem. 242*, 306–311.

Forgac, M., & Cantley, L. (1984) *J. Biol. Chem. 259*, 8101–8105.

Forgac, M., & Berne, M. (1986) *Biochemistry 25*, 4275–4280.

Goffeau, A., & Slayman, C. W. (1982) *Biochim. Biophys. Acta 639*, 197–223.

Jorgensen, P. L. (1975) *Q. Rev. Biophys. 7*, 239–274.

Kyte, J. (1981) *Nature (London) 292*, 201–204.

Linnett, P. E., & Beechey, R. B. (1979) *Methods Enzymol. 55*, 472–518.

MacLennan, D. H., Brandl, C. J., Korczak, B., & Green, N. M. (1985) *Nature (London) 316*, 696–700.

Mellman, I., Fuchs, R., & Helenius, A. (1986) *Annu. Rev. Biochem. 55*, 663–700.

Penefsky, H. S. (1985) *J. Biol. Chem. 260*, 13728–13734.

Rudnick, G. (1986a) *Annu. Rev. Physiol. 48*, 403–413.

Rudnick, G. (1986b) in *Physiology of Membrane Disorders, Second Edition* (Andreoli, T. E., Hoffman, J. F., Fanestil, D. D., & Schultz, S. G., Eds.) pp 409–422, Plenum Press, New York.

Schull, G. E., Schwartz, A., & Lingrel, J. B. (1985) *Nature (London) 316*, 691–695.

Tanford, C. (1961) *Physical Chemistry of Macromolecules*, pp 324–328, Wiley, New York.

Tzagoloff, A., Rubin, M. S., & Sierra, M. F. (1973) *Biochim. Biophys. Acta 301*, 71–104.

Xie, X.-S., & Stone, D. K. (1986) *J. Biol. Chem. 261*, 2492–2495.