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Stabilization of detergent-solubilized Ca^{2+}-ATPase by poly(ethylene glycol)

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The $(Ca^{2+} + Mg^{2+})$-ATPase from sarcoplasmic reticulum (SR) has been solubilized with 1-alkanoyl propanediol-3-phosphorylcholines with chainlengths ranging between 8 and 12 C atoms. A marked dependence of the ATPase activity upon the chainlength was found, indicating that alkyl chainlengths with 12 C atoms are necessary for retention of activity. Addition of poly(ethylene glycol) to the eluting buffers used for gel filtration of the ATPase-detergent micelles was found to increase the activity and the long-term stability significantly. In the presence of Ca^{2+}, the elution volume indicated an ATPase dimer, whereas in the absence of Ca^{2+} the elution volume indicated a monomeric solution. The purity of the preparations after gel filtration was improved by subsequent chromatography with a hydroxyapatite column.

Introduction

In isolated vesicles of sarcoplasmic reticulum, the $(Ca^{2+} + Mg^{2+})$-ATPase contributes 80% of the protein mass [1], and consists of a single polypeptide chain of approximate molecular mass 110 kDa [2]. Models of the protein secondary and tertiary structure show the existence of a hydrophilic region protruding into the cytoplasmic space which is, in turn fixed via a 'stalk' to a membrane-embedded part, both contributing about half of the molecular mass.

According to the model proposed by MacLennan et al. [2], the region of the protein located within the lipid bilayer consists of ten membrane-spanning hydrophobic alpha helices and the stalk of five alpha helices which are predominately amphipathic and in which is located the Ca^{2+} binding site. The stalk region takes part in the conformational transition which allows the binding of ATP and the subsequent phosphorylation of the protein. These transitions may, in turn, provide the mechanism whereby calcium ions are transported through the membrane structure.

Although, in their natural environment, integral proteins are normally surrounded by lipid molecules, it is possible to solubilize some protein in active form using special detergents [3–7]. The preparation of soluble, active forms of integral membrane proteins is of considerable value in physical studies of protein structure and function, it offers for example the potential of leading to the formation of protein-detergent crystals for use in X-ray crystallography [8]. In the case of the $(Ca^{2+} + Mg^{2+})$-ATPase, it has been shown that the polyoxyethylene detergents are very good at maintaining the activity of the enzyme after its removal from the lipid bilayer [4,5,7]. It, however, remained surprising that in order to get the enzyme active in micellar solution, these detergents had to be added in concentrations much higher than usually necessary to saturate membrane proteins with detergents.

We have therefore investigated the effect of adding the polyoxyethylene as a polymer to the solution and using a detergent with a different headgroup for solubilization. As the enzyme in its native membrane environment interacts with phospholipids, we tried the chemically similar 1-alkanoyl propanediol-3-phosphorylcholines.

Abbreviations: cmc, critical micellar concentration; ES-*n*-H, 1-alkanoyl propanediol-3-phosphorylcholine; ET-*n*-H, 1-alkoxy propanediol-3-phosphorylcholine; PEG, poly(ethylene glycol); SR, sarcoplasmic reticulum; C_8E_4, tetraethylene glycol monooctyl ether; $C_{12}E_8$, octaethylene glycol monododecyl ether; OG, *β*-ω-D-octylglucopyranoside.

Materials and Methods

Preparation and purification of SR ($Ca^{2+} + Mg^{2+}$)-ATPase (SR vesicles)

Sarcoplasmic reticulum was prepared from the back and hind-leg muscle of rabbits. The muscle was quickly removed and dissected free of connective tissue before being minced and then homogenized in ice-cold buffer (0.1 M KCl, 10 mM Tris-HCl (pH 7.3)).

The homogenate was centrifuged at $2000 \times g$ for 15 min to sediment the cell debris, after which the supernatant was collected and the pellets rehomogenized in fresh buffer. After centrifuging the second homogenate as described above, the supernatant was collected and pooled with that obtained from the first centrifugation. The combined supernatants were then centrifuged at $10000 \times g$ for 15 min and the pellets discarded before sedimentation of the microsomal fraction was performed at $40000 \times g$ for 1 h. The microsomal pellets were resuspended in 0.6 M KCl, 10 mM Tris-HCl (pH 7.3) and incubated on ice for 10 min, after which they were centrifuged at $100000 \times g$ for 40 min. The SR pellets were finally resuspended in sucrose buffer (250 mM sucrose, 1 M KCl, 2 mM dithiothreitol, 50 mM Tris-HCl (pH 8.0)) and stored frozen.

Purification of the ($Ca^{2+} + Mg^{2+}$)-ATPase (ATP: phosphohydrolase, EC 3.6.1.3) was performed according to the method of Warren et al. [9] using 0.5 mg cholate per mg protein.

Detergents

1-Alkanoyl propanediol-3-phosphorylcholines, or 1-alkanoyl-2-deoxy phosphorylcholines, called ES-*n*-H in the following, as well as 1-alkoxy propanediol-3-phosphorylcholines, or 1-alkoxy 2-deoxyphosphorylcholines, called ET-*n*-H in the following, were synthesized according to Refs. 10–14 or obtained from R. Berchtold, Biochemisches Labor, Bern, Switzerland and synthesized according to Hirt and Berchtold [15]. The abbreviations were introduced by Weltzien et al. [16] and refer to the ester and ether bonds, respectively. The cmc values were determined by the surface tension method [17] using a surface tensiometer (Rosano, Biolar, U.S.A.). Lauryldimethylamineoxide (purity > 97%) was purchased from Fluka, Neu Ulm, F.R.G., OG and C_8E_4 were from Bachem, Heidelberg, F.R.G., laurylmaltoside was from Boehringer Mannheim, Mannheim, F.R.G. and octanoyl-*N*-methylglucamide was from Oxyl, Bobingen, F.R.G.

Analytical methods

SDS gel electrophoresis was performed according to the method of Laemmli [18].

The ATPase activity was determined with a coupled enzyme assay [19] with the exception that 0.1 M imidazole (pH 7.4) was used instead of triethanolamine buffer and the NADH concentration in the assay medium was increased to 0.4 mM. In addition, the detergent was included in the assay medium at the desired concentration. The rate of oxidation of NADH was monitored by recording at 340 nm, using an absorption coefficient of $6.22 \cdot 10^6$ $cm^2 \cdot mol^{-1}$ for NADH [20]. In the case of the screening tests, SR vesicles with between 10 and 30 μg of protein were added to the assay medium and the activity was monitored immediately, i.e., the Ca^{2+}-independent activity was not determined. The protein concentration was determined using the method of Lowry et al. [21] and the international activity units calculated according to the formula: I.U. = μmol of turnover of ATP per mg ATPase per min.

Chromatographic methods

All chromatographic separations were made using a Pharmacia FPLC equipped with two P500 piston pumps and a home-made controller allowing for the mixing of linear binary gradients. The elution profile was determined by measurement of the absorption at 280 nm with a UV1 monitor (Pharmacia).

Buffers. Buffer B1: to 25 mM imidazole-CH_3COOH (pH 7.4) is added glycerol (99% pure, Roth, F.R.G.) to a final concentration of 25% w/v. To this solution either 6% w/v PEG1000 (Merck, F.R.G.), 8% w/v PEG400 (Merck) or 6% w/v PEG4000 is added and finally it is adjusted to 0.3 M potassium chloride, 2 mM sodium azide, 2 mM glutathione and 0.035% w/v of ES-12-H.

Buffer B2: to a sodium phosphate buffer of desired phosphate concentration and of pH 6.8, all additives are given as described for B1.

'glyc' solution: to 10 ml of glycerol, were added 3 ml of H_2O. Finally, either 400 μM of $CaCl_2$ or 750 μM of EGTA was added.

Small-scale gel filtration with superose 6 prep grade. Superose 6 prep grade (Pharmacia) was packed following the instructions supplied by the producer into a gel bed with a diameter of 1 cm and a height of 38 cm. The column was equilibrated and run at a flow rate of 0.15 ml/min. The eluate was collected in fractions of 0.5 ml. For solubilization, 40 μl of SR vesicles (6 mg/ml), 20 μl of 'glyc' solution and 26 μl of a 10% w/v stock solution of ES-12-H in water were mixed. After incubation for 30 min at room temperature, 60 μl of 'glyc' solution were added again and the solubilized protein was loaded to the column.

Preparative gel filtration. Superose 6 prep grade (Pharmacia) was packed into a gel bed with a diameter of 1.6 cm and a height of 64 cm. The column was equilibrated and run at a flow rate of 0.15 ml/min either in buffer B1 or B2. For estimating the molecular weight of the ($Ca^{2+} + Mg^{2+}$) ATPase-detergent micelle, the elution volumes of albumin (bovine, $M_r = 67000$), aldolase ($M_r = 158000$), catalase ($M_r = 232000$), ferri-

tin (M_r = 440 000) thyroglobulin (M_r = 669 000) and blue dextran (M_r = 2 000 000) were determined. Albumin was from Sigma, all other proteins were obtained from Pharmacia, Freiburg, F.R.G.). For calibrating the column, the logarithm of the molecular weight of each protein was plotted against $K_{av} = (V_e - V_0)/(V_T - V_0)$. V_e = elution volume, V_T = volume of the gel bed, V_0 = void volume [22].

Hydroxyapatite chromatography. Hydroxyapatite chromatography was either done with HA ultrogel (Serva Feinbiochemica) packed into a gel bed of a diameter of 0.9 cm and a height of 13 cm and eluted at a flow rate of 0.5 ml/min, or with a Bio-Gel HPHT column set (Bio-Rad Laboratories, München, F.R.G.) eluted at a flow rate of 0.15 ml/min. Both hydroxyapatite columns were equilibrated with a buffer made by adding the desired $CaCl_2$ or EGTA concentrations to B2. For gradient mixing, two B2 buffers were made up from the desired initial and final phosphate concentrations respectively, with the desired concentrations of Ca^{2+} or EGTA.

Solubilization of SR vesicles prior to chromatography. 100 µl of SR vesicles with a protein concentration of some 25 mg/ml were diluted with 200 µl of the buffers B1 or B2 containing Ca^{2+} or EGTA in the same concentration as the elution buffer used to equilibrate the column. Then, 150 µl of the corresponding 'glyc' solution were added. For solubilization 42 mg ES-12-H were added. Care was taken that the detergent dissolves and the solution was incubated at room temperature for 30 min. Finally, 190 µl of the aforementioned elution buffers were added and the solution was applied to the column.

Results

Screening experiments

The ATPase activity of SR vesicles typically was between 9 and 15 I.U.

In screening experiments, various detergents were tested for their ability to keep the ATPase active. The following observations were made: with the detergent present in the assay medium at concentrations far below its cmc the ATPase was active (see Fig. 1). Close to the cmc, or at concentrations corresponding to between 1- and 3-times that of the cmc, the rate of ATP hydrolysis dropped to zero within 2 or 3 min. After addition of new SR vesicles, the same time-course was observed, showing that the ATPase activity has disappeared while the assay reaction remained unchanged (see Fig. 1).

The following detergents caused this type of ATPase inactivation: OG, laurylmaltoside, lauryldimethylamineoxide, octanoyl-*N*-methylglucamide, C_8E_4 and the series of ether derivatives ET-*n*-H with n = 10,12.

With the corresponding ester derivatives, ES-*n*-H, no quick inactivation within 1 or 2 min was observed, but a

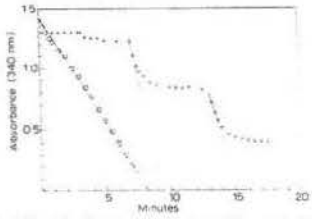


Fig. 1. Plot of the difference of the absorbance of the assay medium at 340 nm and a cuvette filled with water as a function of time. ○, 8 µg of SR vesicles were added to 3 ml of an assay medium containing 0.3% w/v of OG; +, the assay medium contained 0.6% w/v OG, which is equivalent to the cmc. 8 µg of SR vesicles were added after 3 min. 32 µg were added after 7.5 and 12.5 min.

reduction in activity occurred at higher detergent concentrations. In Fig. 2, the ATPase activity is shown as a function of detergent concentration for ES-*n*-H (n = 8, 10, 11, 12). When comparing the different chainlengths, one has to take into account the different cmc values (see Table I). For n = 9 and 10, the cmc values are close to the end of the decreasing region of the plot of detergent concentration versus the activity of the corresponding detergent. For n = 11, the cmc is at a concentration where the activity forms a plateau. For n = 12, the activity is constant over a wider concentration range as compared to all the previous chainlengths, with the cmc at a concentration where the activity is still increasing.

Chromatographic experiments

Small-scale gel filtration as described under Materials and Methods was done in order to elaborate the

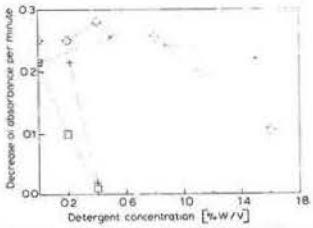


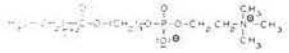
Fig. 2. Decrease per minute in the absorbance at 340 nm after addition of 8 µg of SR vesicles to 3 ml of the assay medium plotted as a function of the detergent concentration in the assay medium. ◇, ES-8-H; □, ES-10-H; +, ES-11-H; ×, ES-12-H. The cmc values are given in Table I.

TABLE I

cmc values of the ES-n-H and the ET-n-H series given as %w/v

	ES-n-H [a]	ET-n-H [b]
$n = 8$	1.8	-
$n = 10$	0.17	0.076
$n = 11$	0.02	0.016
$n = 12$	0.016	0.010

a



b

optimal elution buffers. The elution volume as well as the width of the eluting ATPase band was dependent upon the type of PEG added to the elution buffer. In preliminary experiments, the maximal concentration of PEG400, PEG1000 and PEG4000, which did not increase the elution volume, was determined to be 8, 6 and 6% w/v, respectively. The results are shown in Table II. The addition of PEG to the elution buffer was found to increase the activity of the eluting ATPase by a factor of 7. Glycerol as well as DTT were found to be

TABLE II

Influence of additives to the elution buffer on the activity of the eluting ATPase

40 μl of ATPase vesicles were solubilized and subjected to gel filtration with Superose 6B. The activity is given as the decrease of absorbance at 340 nm/min of the test medium after addition of 100 μl of the 'peak fraction' (fraction sample with maximal protein concentration). As the peak fractions do not contain identical amounts of protein, due to varying locations of the peak fraction relative to the absorbance maximum, as well as differences in the width of the elution profile, an error of the activities of up to 30% was estimated, which is reflected in the standard deviations (S.D.) indicated in the Table. The elution buffer was made from 25 mM imidazole-CH_3COOH (pH 7.4) to which additives were added (glycerol, 2.5 M; PEG or ethyleneglycol; DTT, 1 mM). Finally, the elution buffer was adjusted to 1 mM NaN_3, 0.3 M KCl, 0.1 mM $CaCl_2$. 0.035% w/v ES-12-H. The standard deviation as well as the number of the experiments are given.

Additives	A_{340nm}/min		Number of expts.
	mean	S.D.	
DTT, glycerol	0.041	0.04	6
Glycerol	0.075	0.03	4
DTT, glycerol, PEG400	0.44	-	1
DTT, glycerol, PEG1000	0.27	0.07	3
DTT, glycerol, PEG4000	0.29	-	1
Glycerol, PEG4000	0.07	-	1
DTT, glycerol, ethylene glycol	0.0	-	1
DTT, PEG400	0.0	-	1

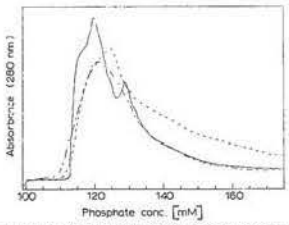


Fig. 3. Hydroxyapatite chromatography of solubilized SR vesicles. The column was loaded with solubilized SR vesicles and eluted with a linear phosphate gradient in the presence of 50 or 250 μM Ca^{2+}. HPLC hydroxyapatite set (Bio-Rad, HPHT) in the presence of 50 or 250 μM Ca^{2+} (———); a column packed with HA-Ultrogel in the presence of 50 or 250 μM Ca^{2+} (- - - - -); a column packed with HA-Ultrogel without Ca^{2+}, but with 500 μM EGTA added (– · –).

necessary additives to retain this high activity. With increasing degree of polymerization of PEG the width of the elution band increased. The resulting difference in protein concentration for the different PEGs possibly explains the variation of the rate of ATP hydrolysis in the experiments where DTT, glycerol and PEG were present. The addition of 8% PEG400 gave the highest ATP hydrolysis rate.

For the following gel filtration experiments, these conditions were used on 'preparative scale', as described under Materials and Methods.

In buffer B2 with 10 mM phosphate buffer and with 50 or 250 μM $CaCl_2$ or 500 μM EGTA added, the protein could be loaded to either the HA ultrogel or the HPHT HPLC column. Either of the two hydroxyapatite columns was then eluted with two successive linear phosphate gradients in B2. The first gradient ascends from 10 to 100 mM phosphate, the second from 100 to 250 mM. No protein eluted during the first gradient. In all cases, the ATPase eluted at phosphate concentrations between 100 and 160 mM. The elution profiles obtained with 50 or 250 μM $CaCl_2$ in the elution buffer and using the column packed with HA ultrogel and the HPHT column set are shown in Fig. 3. With both columns, the overall shape of the elution profile is similar, but the HPHT set shows higher resolution. While the ultrogel column shows a shoulder at 118 mM, a maximum at 125 mM and a tail off around 142 mM, the HPLC column shows peaks at 116, 121 and 128 mM phosphate.

When 500 μM EGTA is present in the equilibration as well as all elution buffers of the gradient, a different elution profile is obtained with the ultrogel column, which is shown in Fig. 3. A maximum is observed at 120 mM and a shoulder at 128 mM. A tail off was observed at 140 mM.

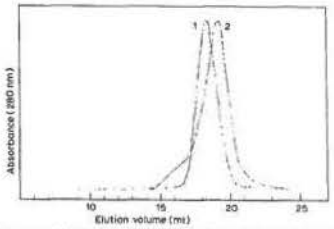


Fig. 4. Gel filtration of solubilized SR vesicles. In the presence of 50 μM Ca^{2+} the protein band elutes at 18.2 ml, without Ca^{2+} in the presence of 500 μM EGTA the protein band elutes at 19.0 ml.

The ATPase activity of all major protein fractions eluted from hydroxyapatite columns was between 80 and 100% of the corresponding value for the SR vesicles.

The state of aggregation of the protein eluted in the peak fractions of the ultrogel column in the presence of Ca^{2+} as well as in the presence of EGTA was studied by gel filtration with the Superose 6 prep grade column equilibrated in B2 buffer with Ca^{2+} or EGTA added. In Fig. 4 the elution profiles obtained with Ca^{2+} and with EGTA, respectively, are shown. In the presence of Ca^{2+}, the protein elutes at 18.2 ml, corresponding to a molecular mass of 220 kDa. In the presence of EGTA, the protein elutes at 19 ml, corresponding to a molecular mass of 160 kDa.

The polypeptide composition of the peak fractions obtained by hydroxyapatite chromatography with the ultrogel column and by a single gel filtration with the superose 6 prep grade column were compared by SDS gel electrophoresis. Fig. 5 shows the electrophoretic pattern obtained with SR vesicles, the eluate from the Superose 6 prep grade column and the eluate from the hydroxyapatite column. Aside from a major band at approx. 100 kDa, the eluate from the gel filtration shows a second polypeptide at approx. 90 kDa. This band is absent in the lane loaded with the protein peak eluted from the hydroxyapatite column.

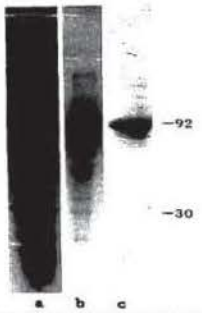


Fig. 5. Electrophoretic pattern obtained with SR vesicles (lane a), the (Ca^{2+} + Mg^{2+})-ATPase preparation obtained by gel filtration (lane b) and by hydroxyapatite chromatography (lane c).

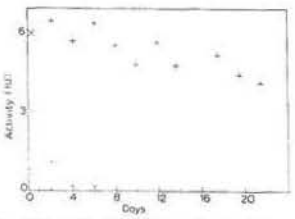


Fig. 6. ATPase activity of the hydroxyapatite preparation as a function of time (for equilibration, buffer B2 was used with 10 mM phosphate and 500 μg M EGTA added and for elution a phosphate gradient was used as given in Results). Values are shown upon storage at 4°C (+) and upon storage at room temperature (×).

Stability of the ATPase preparation

The stability of the ATPase preparation prepared by chromatography with hydroxyapatite was studied by determining the activity every 2 days for material stored at room temperature and at 4°C. Fig. 6 shows that at room temperature the activity decays with a half-life of approx. 1 day, while the decay at 4°C shows a half-life of about 1 month.

Discussion

The screening experiments show that the ATPase has certain requirements with respect to the polar group of the detergent with which it is solubilized. This is consistent with reports by other workers: with desoxycholate it was reported that the ATPase is inactivated when stripping off the lipids beyond a certain minimum of about 4–6 mol lipid bound per mol of protein [23].

The activity can be restored by adding lipids or some detergents to the delipidized protein. Triton X-100 and $C_{12}E_8$ were found to be capable of delipidizing the ATPase to 1–3 mol phospholipid per mol protein [24]. While Triton inactivates the ATPase after a few hours [24,25], the detergent $C_{12}E_8$ does not have this effect when glycerol is present [4].

Dean and Tanford [23] and Dean and Suarez [24] investigated other polyoxyethylene-containing deter-

gents of the Brij and Tween series and found that polar groups with eight to ten ethyleneoxide groups are effective in reactivating delipidated ATPase. They concluded that the size of the polar group should be of importance for the capacity of a detergent to reactivate the ATPase. Our results show that the the headgroup of the 1-alkyl propanediol-3-phosphorylcholines is capable of reactivating the enzyme as well, but that there is also a chainlength requirement for the detergent. A minimum of some 12 C–C bond lengths is necessary to achieve active ATPase. This conclusion is also supported by our results with C_8E_4 and those with Triton mentioned above. Both detergents can be considered short-chain analogues of $C_{12}E_8$ and both inactivate the ATPase.

Dean and Tanford [4] have reported that complete reactivation of the enzyme with polyoxyethylene detergents requires concentrations of the detergent up to 300-times higher than the cmc. In the case of $C_{12}E_8$, this concentration is 20-fold higher than the cmc. On the other hand, membrane proteins are usually saturated with detergent at concentrations between 2- and 3-times the cmc. The polyoxyethylene group thus seems to have some favorable properties which stabilize the (Ca^{2+} + Mg^{2+})-ATPase. Our results show that one can decouple the role of the detergent and the role of polyoxyethylene by adding the latter as a polymeric solution to the buffers which are in contact with the protein. The benefit of decoupling is that one can decrease the detergent concentration to a value 2- or 3-fold that of the cmc.

Our results indicate that the presence of Ca^{2+} during chromatography induces dimer formation. According to our findings, the purity of the ATPase purification can be improved by the use of hydroxyapatite chromatography in place of pure gel filtration. The difference in the elution profile from hydroxyapatite columns eluted with buffers containing $CaCl_2$ and EGTA is possibly explained by the results of the analogous gel filtration experiments. We suppose that the strength of binding of monomers and dimers is different, so that the monomers elute at 118–120 mM phosphate and the dimers at 125–128 mM.

The stability of the ATPase preparation at room temperature is still unsatisfactory. One reason might be the existence of reactive sulfhydryl groups in the (Ca^{2+} + Mg^{2+})-ATPase [26]. To prevent oxidation occurring, glutathione was added to our preparations which we found to be superior to dithiothreitol. Adding fresh glutathione to the preparation every 2 days seems to improve the stability at room temperature. Dux et al. [27] have stored the ATPase under nitrogen to prevent oxidation from occurring.

In accordance with other studies [23], our results show that glycerol improves the stability of the ATPase. Glycerol as well as mono- and disaccharides were found to stabilize other proteins as well. Lee and Timasheff [28], using thermodynamic studies, have predicted that upon addition of these substances to protein solutions, a layer of pure water around the protein would form from which the sugars are excluded. Indeed, Lehmann and Zaccai [29] could prove the existence of a layer of pure water around ribonuclease in the presence of glycerol by neutron small-angle diffraction. The process of denaturation is accompanied by an increase in the surface area of the proteins and consequently in the volume of the free water layer around them. The excess free energy of this volume increase contributes to the stability of the native conformation. An alternative explanation is that the protein is put under additional pressure due to an increase in its surface free energy in the presence of the repelled substance.

Repulsive interaction phenomena are also known of aqueous solutions of two polymers. Phase separation is found in aqueous solutions of PEGs and dextrans [30], while a reduction in the solubility of proteins is found in solutions of proteins and PEGs [31,32]. As discussed by the latter authors, a variety of mechanisms may lead to repulsive interactions. In the case of sugars it may be the repulsion between surface-exposed hydrophobic amino-acid residues and the carbohydrate, while in the case of PEG it may be an excluded volume effect [32], as well as the repulsive interaction of this slightly hydrophobic polymer with charged amino-acid residues as shown by Lee and Lee [33]. In principle, all these repulsive interactions should stabilize the protein according to the same mechanism outlined above for glycerol. However, PEG has a stabilizing effect on some proteins and a destabilizing effect on others, as discussed by Arakawa and Timasheff [32]. The destabilizing effect may be due to the slightly hydrophobic character of PEG, but stabilizing properties could be due to strong repulsive interactions between PEG and clusters of charged amino acids [31,33]. The repulsion is stronger for clusters of negatively charged amino acids, e.g., glutamic acid residues, than for positive ones.

In the model of MacLennan et al. [2], the stalk region, which seems to be critical for the stability of the enzyme, contains many charged amino acids, including a cluster of 16 glutamic acid residues which probably form the Ca^{2+} binding pocket. We think that the stabilizing effect of PEG on the ATPase could be due to strong repulsive interactions of the polymer with the stalk region. The high concentration of $C_{12}E_8$ necessary for obtaining monomeric and active solutions of the ATPase may be a consequence of this unfavourable interaction as well. Swoboda and Hasselbach [35] have studied the ATPase activity after adding detergents to SR vesicles in a phosphatase assay. In this study it was reported that the ATPase loses its activity after solubilization in the presence of Ca^{2+} and/or Mg^{2+} considerably more slowly than in the absence of these divalent ions. The authors conclude that the enzyme is more

stable when the high-affinity binding site for Ca^{2+} is occupied than when it is free. These findings are in accord with our own hypothesis that the stability of the region around the Ca^{2+} binding site is critical for maintaining the ATPase active.

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