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STATE OF AGGREGATION OF DETERGENT-SOLUBILIZED SARCOPLASMIC RETICULUM ADENOSINE TRIPHOSPHATASE INVESTIGATED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The state of aggregation of purified sarcoplasmic reticulum adenosine triphosphatase (ATPase) was investigated by high-performance liquid chromatography (LKB TSK-G 4000 SW column) in the presence of various detergents: sodium dodecylsulphate, dodecyl octaethylene glycol monoether ($C_{12}E_8$), sodium deoxycholate, Triton X-100 and myristoylglycerophosphocholine. When the protein (5 mg ml^{-1}) was solubilized with detergent (2 mg per mg protein) and the column was equilibrated with 1 mg ml^{-1} of the respective detergent, a molecular weight for the monomeric ATPase protein ranging from 100,000 to 200,000 was obtained. In addition to the monomeric form, significant amounts (more than 20%) of aggregated ATPase protein were observed when $C_{12}E_8$ or deoxycholate was used. These results are in agreement with the observation of a great tendency for self-aggregation of the ATPase protein in conventional gel filtration chromatography and ultracentrifugation experiments. The dimeric form of the ATPase protein was detected only when deoxycholate and, probably, when $C_{12}E_8$ was used.

INTRODUCTION

The state of aggregation of sarcoplasmic reticulum ATPase from fast skeletal muscle, solubilized and delipidated with detergents, has been studied by gel filtration[1–7,9–13] and/or by the use of analytical ultracentrifugation[1–3,5–8]. Sodium deoxycholate and $C_{12}E_8$ were the most frequently used detergents, and all reports indicate that the ATPase has a great tendency for self-aggregation. Therefore the mono-, di-, tri- and tetrameric form of the ATPase were detected beside large aggregates which were eluted with the void volume in gel filtration experiments. In contrast, Dean and Tanford demonstrated that the ATPase protein can be obtained as a single monomeric species when delipidated with deoxycholate and solubilized with an excess of $C_{12}E_8$ (2–4 mg per mg protein)[6]. Storage conditions and the amount of detergent used for solubilization seemed to be critical parameters for the state of aggregation

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and could therefore influence the results obtained by conventional gel filtration. We have used high-performance liquid chromatography (HPLC) and a variety of detergents to investigate the distribution between monomeric and oligomeric forms, obtained immediately after solubilization when a completely solubilized ATPase (2 mg detergent per mg protein) was applied. Except when $C_{12}E_8$ or deoxycholate was used, the amount of oligomeric ATPase was less than 10% of the protein and was eluted as large aggregates with the void volume of the column.

MATERIALS AND METHODS

Sarcoplasmic reticulum ATPase was prepared by the method of Hasselbach and König[14] by using sodium deoxycholate for the purification of native vesicles. The protein preparation was diluted to 5 mg ml^{-1} in a buffer, containing 20 m*M* 3-(N-morpholino)propanesulphonic acid (Mops) (pH 7.0) and 50 m*M* sodium chloride. The respective detergent (10 mg ml^{-1}) was added and the solution was filtered through a 1.2-μm filter (Schleicher and Schüll AE 95) prior to application on the HPLC column. It should be noted that less than 10% of protein is lost during filtration. The HPLC column (LKB TSK-G 4000 SW, 600 × 7.5 mm I.D.) was equilibrated in the buffer described above containing different amounts of the respective detergent. The column was operated at a flow-rate of 0.7 ml min^{-1} (20–40 bar) at room temperature and was calibrated with bovine serum albumin (monomer and dimer peak; mol.wt. 67,000 and 134,000, respectively). The calibration was peformed separately for all different detergents at every concentration used. The protein concentration was determined spectrophotometrically at 280 nm ($E^{1\%}_{1cm} = 10$)[1,11]. Sodium dodecylsulphate, myristoylglycerophosphocholine and bovine serum albumin were from Serva (Heidelberg, F.R.G.). $C_{12}E_8$ was from Nikko (Tokyo, Japan), Triton X-100 was from Carl Roth (Karlsruhe, F.R.G.) and sodium deoxycholate from E. Merck (Darmstadt, F.R.G.).

RESULTS

The detergents used in this study are subdivided into three groups: (i) dodecylsulphate, (ii) $C_{12}E_8$ and deoxycholate and (iii) Triton X-100 and myristoylglycerophosphocholine.

Sodium dodecylsulphate

As illustrated in Fig. 1A, the elution pattern of the ATPase protein (5 mg ml^{-1}) solubilized in 10 mg ml^{-1} dodecylsulphate obtained from a column, equilibrated in 20 m*M* Mops (pH 7.0), 50 m*M* sodium chloride and 1 mg ml^{-1} dodecylsulphate shows one major peak representing the ATPase monomer (molecular weight of the protein–detergent micelle: 140,000) (Table I) and minor contaminants with a molecular weight less than 50,000. This elution pattern is comparable with the results obtained by sodium dodecylsulphate gel electrophoresis (Fig. 1A, inset). When the dodecylsulphate concentration in the column buffer was reduced to 0.1 mg ml^{-1} (a concentration below the critical micellar concentration (cmc)), a very different elution profile was obtained. Two distinct peaks and several shoulders are observed, representing aggregated protein species with molecular weights greater than 250,000 (Table I).

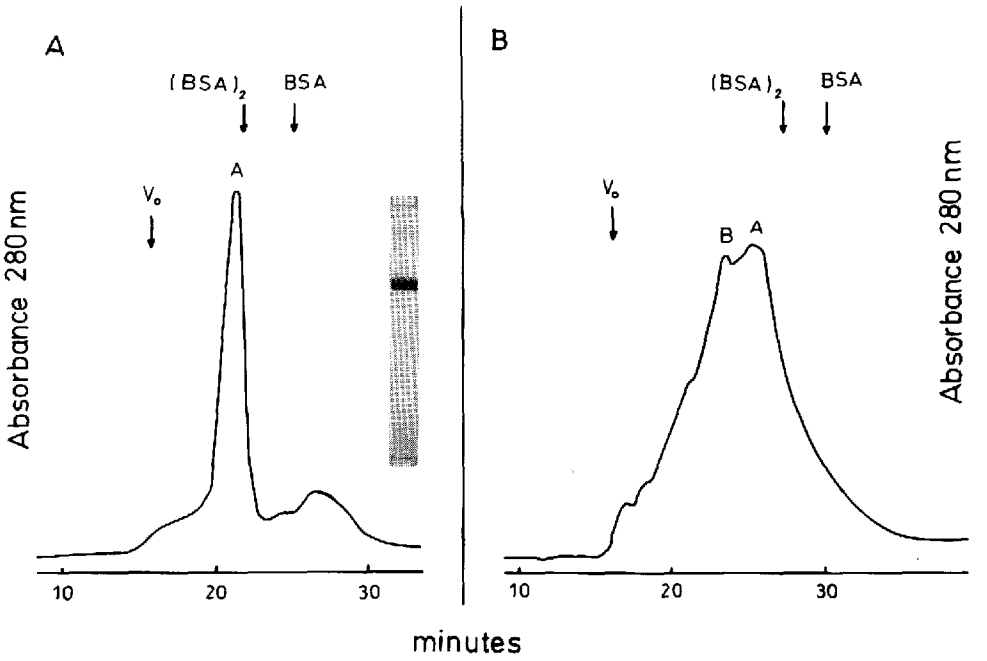

Fig. 1. Elution pattern of ATPase protein solubilized in sodium dodecylsulphate. ATPase protein (5 mg ml^{-1}) was solubilized in 10 mg ml^{-1} sodium dodecylsulphate, and 1 mg of protein was applied to the column (see Materials and Methods). (A) Column equilibrated in 20 m*M* Mops (pH 7.0), 50 m*M* sodium chloride and 1 mg ml^{-1} sodium dodecylsulphate. (B) Column equilibrated in 20 m*M* Mops (pH 7.0), 50 m*M* sodium chloride and 0.1 mg ml^{-1} sodium dodecylsulphate. Inset: Sodium dodecylsulphate gel electrophoresis pattern of the ATPase preparation used for the studies. $(BSA)_2$ = peak of dimeric bovine serum albumin; BSA = peak of monomeric albumin; V_0 = void volume of the column.

TABLE I

MOLECULAR WEIGHTS OF THE DIFFERENT FORMS OF THE ATPASE PROTEIN

The molecular weight determination was performed with bovine serum albumin as a standard. Monomer peak 67,000, dimer peak 134,000: See Materials and Methods.

Detergent	*Concentration in the column (mg ml^{-1})*	*Amount of protein in the void volume (%)**	*Molecular weight*	
			*Peak A***	*Peak B***
SDS***	1	–	145,000§	–
SDS	0.1	–	300,000	500,000
$C_{12}E_8$	1	43	115,000§	160,000
DOC§§	2	16	145,000§	250,000
Triton X-100	1	9	100,000§	–
Myristoylglycero-phosphocholine	1	8	240,000§	–

* Taken as percentage of total protein eluted from the column.
** Compare with figures.
*** Sodium dodecylsulphate.
§ Monomeric ATPase protein.
§§ Sodium deoxycholate.

Sodium deoxycholate and $C_{12}E_8$

If the ATPase protein (5 mg ml^{-1}) was solubilized with either $C_{12}E_8$ or deoxycholate and the column was equilibrated with 1 or 2 mg ml^{-1} of the respective detergent, an elution pattern different from that obtained with 1 mg ml^{-1} dodecylsulphate was observed. A large amount of the protein was eluted with the void volume of the column (Table I) and the protein appeared within two peaks, which in the case of deoxycholate are not well resolved (Fig. 2B). The molecular weights of the two protein species are given in Table I. Peak C in Fig. 2B is attributed to the appearance of deoxycholate micelles, which have an absorbance at 280 nm and a molecular weight less than 23,000. When the concentration of these detergents in the column was reduced below the cmc (0.5 mg ml^{-1} deoxycholate and 0.04 mg ml^{-1} $C_{12}E_8$, respectively) all the protein was eluted with the void volume of the column (not shown; cf. Fig. 3B).

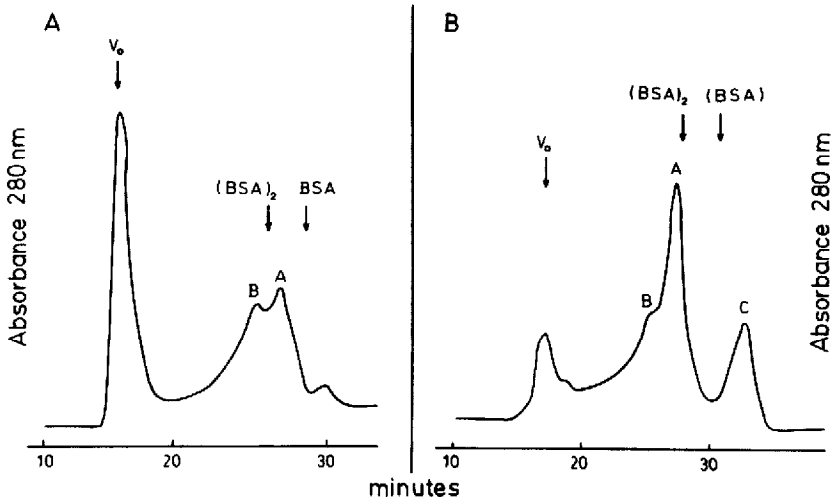

Fig. 2. Elution pattern of ATPase protein, solubilized in $C_{12}E_8$ or sodium deoxycholate. ATPase protein (5 mg ml^{-1}) was solubilized with 10 mg ml^{-1} of $C_{12}E_8$ or sodium deoxycholate. (A) Column equilibrated in 20 m*M* Mops (pH 7.0) 50 m*M* sodium chloride and 1 mg ml^{-1} $C_{12}E_8$. (B) Column equilibrated in 20 m*M* Mops (pH 7.0), 50 m*M* sodium chloride and 2 mg ml^{-1} sodium deoxycholate.

Triton X-100 and myristoylglycerophosphocholine

Results similar to those with dodecylsulphate were obtained when the ATPase protein (5 mg ml^{-1}) was solubilized with 10 mg ml^{-1} Triton X-100 or myristoylglycerophosphocholine. A relatively small amount of the protein (less than 10%) appeared in the void volume of the column whereas the major part was eluted as a single peak (Table I; Fig. 3A and C). If the concentration of Triton X-100 was reduced below the cmc (0.1 mg ml^{-1}) all the protein was eluted with the void volume of the column (Fig. 3B).

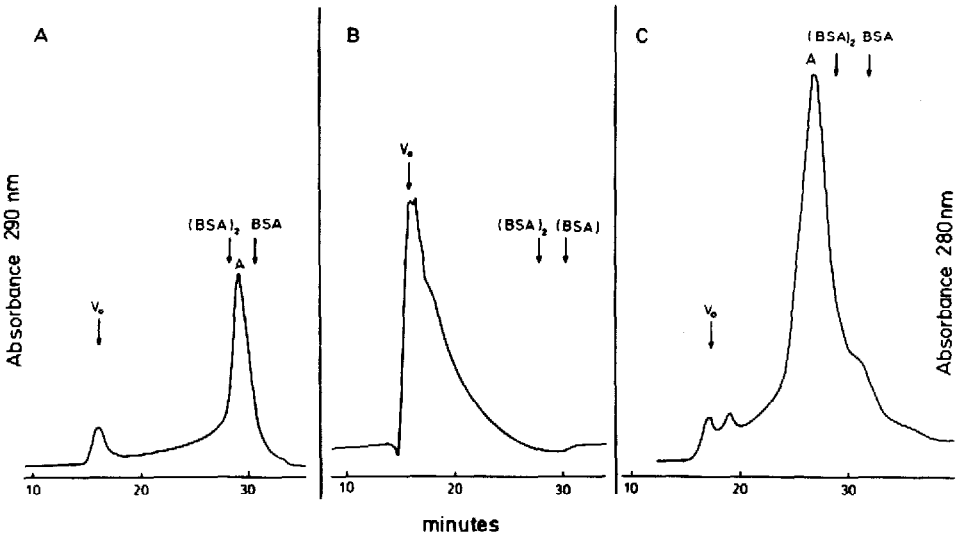

Fig. 3. Elution pattern of ATPase protein, solubilized with Triton X-100 or myristoylglycerophosphocholine. ATPase protein (5 mg ml^{-1}) was solubilized in 10 mg ml^{-1} Triton X-100 or myristoylglycerophosphocholine. (A) Column equilibrated with 20 m*M* Mops (pH 7.0), 50 m*M* sodium chloride and 1 mg ml^{-1} Triton X-100. (B) Same as in A, but the buffer solution contained 0.1 mg ml^{-1} Triton X-100. (C) Column equilibrated with 20 m*M* Mops (pH 7.0), 50 m*M* sodium chloride and 1 mg ml^{-1} myristoylglycerophosphocholine.

DISCUSSION

The results presented in Figs. 1–3 demonstrate that solubilization of the ATPase protein with dodecylsulphate, Triton X-100 and myristoylglycerophosphocholine yields a small amount of large aggregates and predominantly the monomeric form of the ATPase protein. In contrast the results obtained with $C_{12}E_8$ and deoxycholate seem to indicate that a mixture of monomeric and dimeric ATPase is obtained in addition to substantial amounts of large aggregates. (For a detailed description of the results obtained with $C_{12}E_8$ see below.) It should be noted that the ATPase preparation used in this study was delipidated with a small amount of deoxycholate (0.3 mg per mg protein) which was used for the purification and which was removed by dilution and centrifugation. The molecular weight determinations obtained with bovine serum albumin (Table I) are not very accurate and represent the molecular weight of the protein–detergent micelle. But it was our method of choice because albumin binds all the detergents used. The differences obtained for the monomeric form of the ATPase are most probably due to differences in the molecular dimensions of the protein detergent micelle (see, *e.g.* ref. 18). In particular, the molecular weight of the ATPase protein in myristoylglycerophosphocholine seemed to indicate the existence of predominantly dimeric ATPase. However, previous studies[15–17] demonstrated that the peak shown in Fig. 3C represents the monomeric form of the protein. Owing to the short time necessary for one column run (*ca.* 30 min), the results shown in Figs. 1–3 represent the initial distribution of monomeric and oligomeric forms of the completely solubilized ATPase present at the detergent concentration used. As

long as the detergent concentration is above the cmc, no aggregation should take place during a column run (*cf.* Fig. 1A and B and Fig. 3A and B). In addition, no detectable increase of the amount of large aggregates obtained with $C_{12}E_8$ and deoxycholate was observed with three consecutive column runs of the same solubilized ATPase preparation. The results discussed so far are in general agreement with previous reports on the state of aggregation of the ATPase protein in detergent solutions[1–13]. There exist discrepancies with the report of Hidalgo *et al.*[4] where the dimeric and trimeric forms of the ATPase were observed in Triton X-100 solutions, and with the report of Silva and Verjovski-Almeida[13] where under similar conditions the dimeric form of the ATPase was predominantly found in $C_{12}E_8$ solutions. These discrepancies can be explained by differences between the methods used, *i.e.* conventional gel filtration and HPLC.

In contrast to the results obtained with deoxycholate, the molecular weights determined for the monomeric and dimeric forms of the $C_{12}E_8$ solubilized preparation seem to be incorrect, because the dimeric form has a molecular weight only 40% larger than that of the monomeric form. This could be due to (i) differences in the dimensions of the $C_{12}E_8$ micelle containing either the ATPase monomer or the ATPase dimer; or (ii) a chromatography artefact. Indeed, a doubling of the monomer peak was observed when the dodecylsulphate concentration in the column was increased to 2 mg ml^{-1}, for example. This would mean that the peaks A and B shown in Fig. 2A represents the same, most probably monomeric, species of the ATPase protein. On the other hand, it should be noted that the large amount of protein eluted in the void volume from $C_{12}E_8$-equilibrated columns was not a chromatography artefact. Solubilization of the ATPase protein with a $C_{12}E_8$ concentration as large as 40 mg per mg protein did not reduce the amount of protein eluted with the void volume of the column. Furthermore, in contrast to $C_{12}E_8$, when non-solubilized, native ATPase preparations were applied to a column equilibrated with Triton X-100 at a concentration above the cmc, the protein was completely monomerized, *i.e.* the same pattern as shown in Fig. 3A was obtained. As expected, the ATPase protein aggregated when the detergent concentration in the column was reduced to values near or below the cmc. This was also observed with dodecyl sulphate, but the agregates seem too have distinct molecular weights representing the di-, tri-, tetrameric ATPase and possibly larger aggregates, whereas with all other detergents aggregates with a molecular weight greater than 10^6 are obtained.

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