THE DEPENDENCE FOR REACTIVATION OF LIPID-DEPLETED Ca^{2+}-ATPase OF SARCOPLASMIC RETICULUM BY NON-IONIC DETERGENTS ON THEIR HYDROPHILE/LIPOPHILE BALANCE

Vladimir I. MELGUNOV and Elena I. AKIMOVA

Department of Molecular Biology, Faculty of Biology, M. V. Lomonosov State University, Moscow 117234, USSR

Received 3 September 1980

1. Introduction

The Ca^{2+}-ATPase of sarcoplasmic reticulum is an intrinsic membrane protein. It can only be solubilized by disrupting of the membrane with organic solvents or by detergents. At least 25–30 mol phospholipid/mol ATPase polypeptide were believed to be necessary for retention of full enzymatic activity [1]. However, more recently it was shown [2–4] that the enzyme may be obtained in a stable and soluble form, virtually free of phospholipid. In all cases one can obtain an inactive enzyme that can be restored to almost full activity by adding back various phospholipids. Some non-ionic detergents can also efficiently substitute the phospholipids [3]. Here we report a systematic study of the reactivation of Ca^{2+}-ATPase of sarcoplasmic reticulum by various non-ionic detergents and surfactants. The study has been done to determine the conditions under which the enzyme can be restored to full activity. Using cholate–dilution procedure [5], non-ionic detergents studied have been shown to be all able to reactivate completely delipidated Ca^{2+}-ATPase of sarcoplasmic reticulum. Reactivation potency of the detergents used is closely related to their relative hydrophobicity. The latter has been estimated using value of so-called 'hydrophile/lipophile balance' (HLB) number [6]. There is striking correlation between reactivation potency and HLB number of detergent. As the HLB number increases the reactivation potency of the detergent decreases. Correlation coefficient (r) for small samples has been calculated [7] to be equal to -0.95 ± 0.09.

2. Materials and methods

2.1. *Materials*

Tris(hydroxymethyl)aminomethane, dithiothreitol, sodium dodecyl sulfate (SDS) and Lubrol PX were purchased from Sigma (MO). Sephadex G-200, Sephadex G-50 and Sephacryl S-200 were from Pharmacia (Uppsala). Nonidet P40 was from BDH. Tween 21 was a product of Ferak (Berlin). Dioleoyl phosphatidyl choline, dipalmitoyl phosphatidyl choline, Brij 30, Brij 52, Brij 56, Brij 58, Brij 35, Myrj 52, Myrj 59, Span 20, Span 80, Tween 20, Triton X-100, Triton X-305 were purchased from Serva (Heidelberg). Cholic acid (Spofa, Prague or Serva) was recrystallized 3 times from 70% ethanol. All other reagents were from Reanal (Budapest) or Sojuzreaktiv (USSR) and corresponded to analytical or primary standards.

2.2. *Sarcoplasmic reticulum and ATPase preparation*

Rabbit sarcoplasmic reticulum was prepared as in [8]. Ca^{2+}-ATPase was isolated and purified as in [4]. For delipidation, the solubilized ATPase was suspended [2] in 50 mM Tris–HCl (pH 8.0 at 4°C), 0.25 M sucrose, 1 mM histidine, 1 mM dithiothreitol, 1 mM ATP and 1% sodium cholate. The slightly turbid suspension (3 ml of 20 mg protein/ml) was first applied to a Sephadex G-200 or Sephacryl S-200 column (2.5 × 67 cm) equilibrated with the same buffer used for the suspension of the enzyme. To remove cholate, the delipidated ATPase was passed through a Sephadex G-50 column (1.5 × 70 cm) equilibrated with 50 mM Tris–HCl (pH 8.0 at 4°C), 1 mM histidine, 1 mM dithiothreitol, 2 mM ATP and 0.25 M sucrose. The cholate-free solution was usually concentrated to 1.75 mg protein/ml using ultrafiltration on Amicon (Amsterdam) XM-50 membrane.

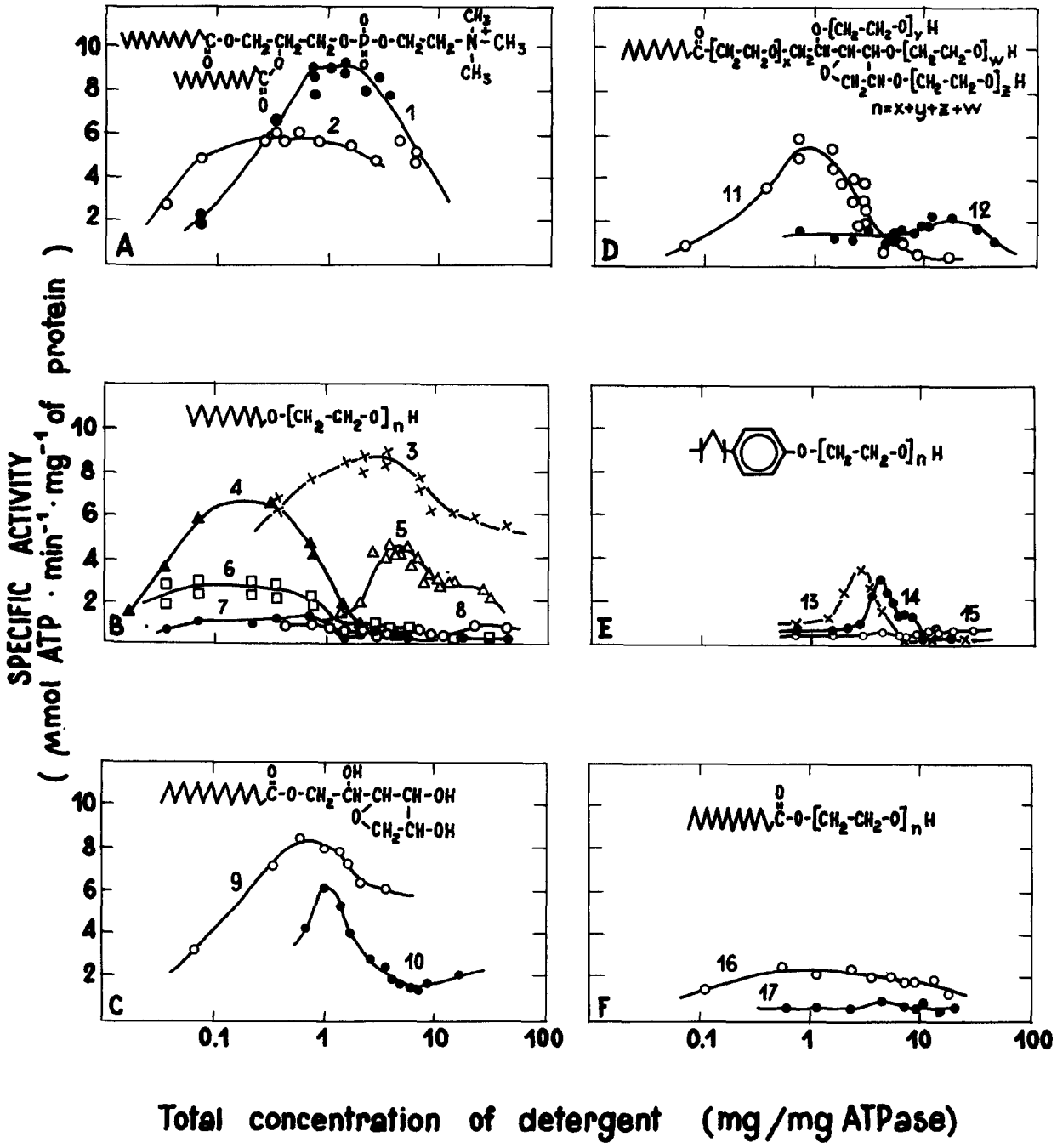

Fig.1. Reactivation of delipidated Ca^{2+}-ATPase by various phospholipids (A), polyoxyethylene alcohols (B), sorbitol esters of fatty acids* (C), polyoxyethylene sorbitol esters* (D), polyoxyethylene *p-tert*–octyl phenols (E) and polyoxyethylene esters (F): 1, dioleoyl phosphatidyl choline; 2, dipalmitoyl phosphatidyl choline; 3, Brij 52 ($C_{16}E_2$); 4, Brij 30 ($C_{12}E_8$); 5, Lubrol PX ($C_{12}E_{9.5}$); 6, Brij 56 ($C_{16}E_9$); 7, Brij 35 ($C_{12}E_{20}$); 8, Brij 58 ($C_{16}E_{20}$); 9, Span 80 ($C_{18:1}$ sorbitol ester); 10, Span 20 (C_{12} sorbitol ester); 11, Tween 21 ($C_{12}E_5$ sorbitol ester); 12, Tween 20 ($C_{12}E_{20}$ sorbitol ester); 13, Nonidet P40 (*p*-tert–octyl phenol E_{10}); 14, Triton X-100 (*p*-tert–octyl phenol $E_{9.5}$); 15, Triton X-305 (*p*-tert–octyl phenol E_{30}); 16, Myrj 52 ($C_{16}E_{33}$ ester); 17, Myrj 59 ($C_{16}E_{95}$ ester); E, oxyethylene residue $-CH_2-CH_2-O-$; *, the formulae shown represent just one type of different possible molecular structures. *n*, av. no. oxyethylene units/molecule.

2.3. *Reactivation of lipid-depleted Ca^{2+}-ATPase*

Delipidated Ca^{2+}-ATPase (140 μg in 80 μl) was mixed with 10 μl 10% sodium cholate (pH 7.95 at 4°C) and 10 μl phospholipid or detergent solution in 96% ethanol (at concentration desired). Total volume of reaction mixture was 100 μl. The mixture was incubated for 3 min at 4°C with continuous mixing on 'Maxi-Mix Vortex Mixer' (Thermolyne Corp.). Aliquots (70 μl) were used for standard Ca^{2+}-ATPase assay at 37°C as in [8].

2.4. *Other methods*

Phospholipid phosphorus in protein samples was measured according to [9]. Protein concentrations were determined as in [10]. Polyacrylamide gradient gel electrophoresis was performed as in [11].

3. Results

The gel-filtration on Sephadex G-200 or Sephacryl S-200 removes almost 90% of phospholipid present initially in ATPase preparations. Residual (10%) phospholipids are separated from the protein during the cholate removal step. This so-called 'cholate-free' [2] preparation does not contain phospholipid phosphorus and has no enzymatic activity [4].

Fig.1A shows that ATPase activity can be completely restored by reactivation in the presence of phosphatidyl choline and sodium cholate. ATPase activity obtained is Ca^{2+}-dependent; it is completely inhibited by ethylene glycol-bis-(2-amino ethyl ether) *N,N'*-tetraacetate and is partially recovered after subsequent addition of $CaCl_2$. Reactivation of the enzyme is due to exchange of cholate present in protein–detergent complex onto membrane-forming diacyl phospholipids. Reactivation efficiency of phospholipid is determined by the composition of fatty acids. It increases together with the unsaturation extent of fatty acids.

As the native lipid environment of Ca^{2+}-ATPase is very complex we tried to simulate it by means of various detergents. The detergents used fall into five classes: (1) polyoxyethylene alcohols; (2) polyoxyethylene sorbitol esters; (3) polyoxyethylene *p*-tert-octyl phenols; (4) polyoxyethylene esters; and (5) sorbitol esters of fatty acids. The concentration dependences of reactivation potency of various detergents are represented in fig.1B–F. The detergent concentration is plotted on a logarithmic scale for convenience. One can see that essentially the same level of Ca^{2+}-ATPase activity can be restored when exogenous phospholipids are replaced by non-ionic detergents such as Brij 52 and Span 80. The reactivation extent in the case of Brij 30, Span 20 and Tween 21 does not exceed 60%. Detergents which are widely used for solubilization of the membrane enzymes such as Triton X-100, Lubrol PX and Nonidet P40 can reactivate the lipid-depleted ATPase only to 30–40%. Some reactivation is observed also in the presence of Brij 56, Brij 58, Brij 35, Myrj 52, Myrj 59, Tween 20 and Triton X-305.

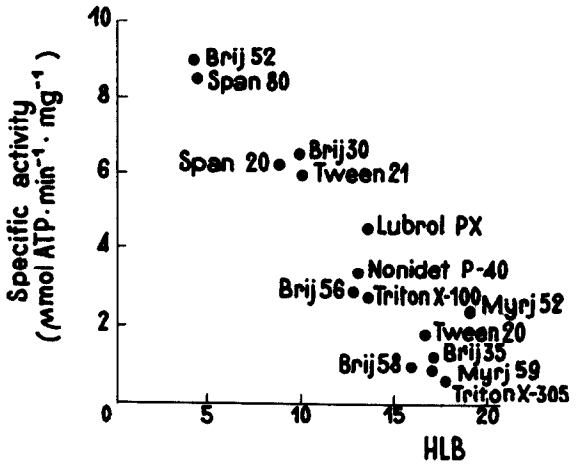

Fig.2. HLB dependence of reactivation potency of various detergents and surfactants. HLB values for non-ionic detergents and surfactants have been calculated from the average molecular structure as in [6].

The maximal reactivation by any detergent was shown (fig.2) to depend on its relative hydrophobicity. As an estimate of the relative hydrophobicity of the detergents used, we used the so-called HLB number [6]. This empirical measure allows one to compare the relative sizes of the polar head group and hydrophobic hydrocarbon chain. It should be remarked that the points shown in fig.2 lie very nearly on a straight line, i.e. there is striking correlation between the reactivation potency and HLB number of the various detergents. The inverse correlation coefficient (r) was calculated to be equal to -0.95 ± 0.09.

4. Discussion

The regularity found above is quite different from those [12,13] described for solubilization of several

membrane enzymes where the solubilizing potency *vs* HLB number curve is bell-shaped and has a distinct maximum. Almost all effective detergents were shown to be in 12.5–14.5 HLB range.

On the other hand it appears from the information of fig.2 that as the HLB increases, polarity increases, the degree of hydration increases, the critical micelle concentration increases, the detergent micelle size decreases [14] and the reactivating potency also decreases. Sodium cholate added to the reaction mixture causes a significant disordering of hydrocarbon chain and head group organization and favours the effective formation of protein–detergent complexes. The orientation of the protein in two different phases (lipophilic and hydrophilic) seems to be restored during reactivation and in many cases the protein-bound detergent mimics the lipid environment in the membrane sufficiently well to support continued activity of the Ca^{2+}-ATPase. For some time is has been generally thought that there is a special class of 'ordered' or 'immobilized' lipid molecules which are in very close contact with protein molecules. When the so-called 'annular' [1], 'halo' [15] or 'boundary' [16] lipids are removed, an enzyme such as Ca^{2+}-ATPase is believed to be irreversibly inactivated. Bile salts have an enormous capacity [14] to solubilize swelling amphiphiles, they are very effective for removal of such residual tightly-bound lipids. The use of various stabilizers (e.g., glycerol or sucrose) helps one to bypass the problem of irreversible inactivation and to obtain stable and soluble preparations of the enzyme.

As far as the 'boundary' lipids can be efficiently replaced not only by natural phospholipids but also by various non-ionic detergents one can assume that these tightly bound lipids do not determine per se the functioning Ca^{2+}-ATPase of sarcoplasmic reticulum and they are necessary only to create a hydrophobic environment near the enzyme itself.

Acknowledgements

We acknowledge with gratitude and affection the generous help we have received from Professor A. S. Spirin. We are grateful to Miss N. M. Goreyshina for expert preparation of the manuscript.

References

[1] Warren, G. B., Houslay, M. D., Metcalfe, J. C. and Birdsall, N. J. M. (1975) Nature 255, 684–687.
[2] Knowles, A. F., Eytan, E. and Racker, E. (1976) J. Biol. Chem. 251, 5161–5165.
[3] Dean, W. L. and Tanford, C. (1977) J. Biol. Chem. 252, 3551–3553.
[4] Melgunov, V. I. and Akimova, E. I. (1978) Dokl. Akad. Nauk SSSR 238, 1487–1489.
[5] Racker, E., Chien, T.-F. and Kandrach, A. (1975) FEBS Lett. 57, 14–18.
[6] Griffin, W. C. (1949) J. Soc. Cosmet. Chem. 1, 311–326.
[7] Snedecor, G. W. (1961) Statistical methods applied to experiments in agriculture and biology, pp. 161–167, Russian transl., Moscow.
[8] Ritov, V. B., Melgunov, V. I., Komarov, P. G., Alexeeva, O. M. and Akimova, E. I. (1977) Dokl. Akad. Nauk SSSR 233, 730–733.
[9] Kulaev, I. S. and Melgunov, V. I. (1973) *Neurospora* Newslett. no. 20, 35.
[10] Schaffner, W. and Weissman, C. (1973) Anal. Biochem. 56, 502–514.
[11] Melgunov, V. I. and Akimova, E. I. (1980) FEBS Lett. 111, 197–200.
[12] Umbreit, J. N. and Strominger, J. L. (1973) Proc. Natl. Acad. Sci. USA 70, 2997–3001.
[13] Storm, D. R., Field, S. O. and Ryan, J. (1976) J. Supramol. Struct. 4, 221–231.
[14] Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79.
[15] Stier, A. and Sackman, E. (1973) Biochim. Biophys. Acta 311, 400–408.
[16] Marsh, D., Watts, A., Mashke, W. and Knowles, P. F. (1975) Biochem. Biophys. Res. Commun. 81, 397–402.