## BBA Report

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# Lipid composition of purified fragmented sarcoplasmic reticulum of the rabbit

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SUMMARY

Phospholipids were assayed in fragmented sarcoplasmic reticulum and the R3d fraction prepared from fragmented sarcoplasmic reticulum as described by MacLennan (MacLennan, D.H. (1970) *J. Biol. Chem.* 245, 4508–4518). The fragmented sarcoplasmic reticulum contained 9.2% phosphatidylinositol and the R3d fraction, 9.4%. Ethanolamine plasmalogen was more than two-thirds the amount of ethanolamine-containing phospholipid in both fractions. A 4-fold increase in activity of $Ca^{2+}$-ATPase (3.8 to 16.8 μmoles $P_i$/mg protein per min) did not produce an enrichment in any single phospholipid.

The technique of MacLennan[1] has enabled a 10-fold increase in activity of the $Ca^{2+}$-ATPase of fragmented sarcoplasmic reticulum from skeletal muscle (3.0–30.8 μmoles $P_i$/mg protein per min). In a subsequent publication[2] the lipid composition of the purified $Ca^{2+}$-ATPase was reported, indicating an absence of inositol-containing phospholipids and a plasmalogen content of approximately one-third of the total ethanolamine-containing phospholipids. Meissner and Fleischer[3] found 9% phosphatidylinositol in fragmented sarcoplasmic reticulum of rabbit with a $Ca^{2+}$-ATPase activity 0.9 μmoles $P_i$/mg protein per min in the absence of detergent and 3.5 μmoles $P_i$/mg protein per min in the presence of 0.05% deoxycholate; these authors did not analyze plasmalogen phospholipids. In contrast, Waku *et al.*[4] have reported a phosphatidylinositol content of approximately 2% and the major part of the ethanolamine phospholipid was plasmalogen.

The present study was undertaken to determine whether enrichment of specific phospholipids might be correlated with purification of the $Ca^{2+}$-ATPase of fragmented sarcoplasmic reticulum.

Sarcoplasmic reticulum and purified $Ca^{2+}$-ATPase (R3d) were prepared by the method described by MacLennan[1] using albino rabbits weighing 5–10 lb. Skeletal muscle from the hind legs and back was extracted with buffer containing 120 mM NaCl and 5 mM imidazole, pH 7.4. The material sedimented between 10 000 × *g* and 40 000 × *g* was taken as the crude sarcoplasmic reticulum (R1, according to the nomenclature of MacLennan). This was suspended in the extraction buffer at a protein concentration of 10 mg/ml and the material sedimenting at 10 000 × *g* was discarded. The fraction sedimenting between 10 000 × *g* and 78 000 × *g* was taken as the washed sarcoplasmic reticulum. In one case the washing step was omitted and the crude sarcoplasmic reticulum was used to obtain the R3d fraction. The crude sarcoplasmic reticulum was extracted with twice-recrystallized deoxycholate in the presence of 1 M KCl to obtain the R2 fraction which was further treated with deoxycholate and ammonium acetate to obtain the R3d fraction.

Total lipid extracts were obtained from the various suspensions by homogenization in 20 vol. of ice-cold chloroform–methanol (2:1, v/v) containing butylated hydroxytoluene (5 mg/100 ml). After heating at 50 °C for 5 min, the suspensions were cooled to room temperature and filtered through sintered glass. The extracts were washed with 0.1 M KCl, dried under reduced pressure and redissolved in chloroform–methanol (2:1, v/v).

Phospholipid composition was determined by two-dimensional thin-layer chromatography in the presence of mercuric chloride[5].

Table I lists the percentage of phospholipid phosphorus for fragmented sarcoplasmic reticulum and the R3d fraction of the $Ca^{2+}$-ATPase. The plasmalogen content of the total choline and ethanolamine-containing phospholipids are presented. The data for fragmented sarcoplasmic reticulum represent the mean ± S.E. of three experiments. The lipid analysis of the R3d fraction represents the composition of the preparation with the highest specific activity attainable in our laboratory (16.8 μmoles $P_i$/mg protein per min).

TABLE I

PHOSPHOLIPID COMPOSITION (% OF TOTAL LIPID-P) OF FRAGMENTED SARCOPLASMIC RETICULUM AND PURIFIED $Ca^{2+}$-ATPase OF RABBIT SKELETAL MUSCLE

| *Phospholipid* | *Sarcoplasmic reticulum* | *$Ca^{2+}$-ATPase* |
|---|---|---|
| Choline phospholipid (total) | 67.0 ± 1.5 | 63.9 |
| Choline plasmalogen | 8.1 ± 2.1 | 10.7 |
| Ethanolamine phospholipid (total) | 17.6 ± 0.8 | 20.4 |
| Ethanolamine plasmalogen | 13.3 ± 0.7 | 16.3 |
| Phosphatidylinositol | 9.2 ± 0.3 | 9.4 |
| Phosphatidylserine | 2.1 ± 0.6 | 2.9 |
| Sphingomyelin | 3.9 ± 1.4 | 3.1 |
| Cardiolipin | 0.1 ± 0.1 | 0 |
| $Ca^{2+}$-ATPase (μmoles $P_i$/mg protein/min | 3.8 ± 0.8 | 16.8 |

Our results for phospholipid composition of fragmented sarcoplasmic reticulum agree closely with those of Meissner and Fleischer[3]. These authors did not report plasmalogen content of phospholipids. The percentage of total choline phospholipid (64.9%, fragmented sarcoplasmic reticulum; 65.9%, $Ca^{2+}$-ATPase) and total ethanolamine phospholipid (18.8%, fragmented sarcoplasmic reticulum; 17.4%, $Ca^{2+}$-ATPase) reported by MacLennan *et al.*[2] are almost identical to our values. However, the plasmalogen component of their ethanolamine phospholipid was about 33% in both preparations, whereas in our results the plasmalogen was about 75% of the total ethanolamine phospholipid. In agreement with the present studies, Waku *et al.*[4] have reported choline and ethanolamine plasmalogen components of 9.5 and 69.3%, respectively for fragmented sarcoplasmic reticulum of rabbit.

Another striking difference was the presence of phosphatidylinositol (9.5%, fragmented sarcoplasmic reticulum; 9.4%, $Ca^{2+}$-ATPase) in accord with Meissner and Fleischer[3]. It may also be mentioned that Sanslone *et al.*[6] reported 9.3% phosphatidylinositol in rat skeletal muscle fragmented sarcoplasmic reticulum phospholipid. The presence of phosphatidylserine (11.4%, fragmented sarcoplasmic reticulum; 11.6%, $Ca^{2+}$-ATPase) in the analyses of MacLennan *et al.*[2] most probably represents inositol *plus* serine phospholipid that was insufficiently resolved by chromatography. However, in agreement with MacLennan *et al.*[2] no enrichment in specific phospholipids could be correlated with an increase in the specific activity of the $Ca^{2+}$-ATPase relative to fragmented sarcoplasmic reticulum of skeletal muscle.

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REFERENCES

1 MacLennan, D.H. (1970) *J. Biol. Chem.* 245, 4508–4518
2 MacLennan, D.H., Seeman, P., Iles, G.H. and Yip, C.C. (1971) *J. Biol. Chem.* 246, 2702–2710
3 Meissner, G. and Fleischer, S. (1971) *Biochim. Biophys. Acta*, 241, 356–378
4 Waku, K., Uda, Y. and Nakazawa, Y., (1971) *J. Biochem. Tokyo* 69, 483–491
5 Owens, K. (1966) *Biochem. J.* 100, 354–361.
6 Sanslone, W.R., Bertrand, H.A., Yu, B.P. and Masoro, E.J. (1971) *J. Cell Physiol.* 79, 97–102