RECONSTITUTION OF AN EFFICIENT CALCIUM PUMP WITHOUT DETERGENTS

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SUMMARY

A calcium pump has been reconstituted with purified phospholipids and an ATPase from sarcoplasmic reticulum by sonication without detergents. With the proper mixture of phospholipids and prolonged sonication vesicles were formed with low ATPase and efficient calcium transport activity. By increasing the ratio of protein to phospholipids the rate of calcium transport decreased but became responsive to stimulation by externally added oxalate. It has thus become possible to analyze by variations in reconstitution some of the characteristic features of the sarcoplasmic reticulum.

A calcium pump was previously reconstituted (1) by combining a preparation of the Ca^{++} ATPase of sarcoplasmic reticulum from rabbit muscle (2) with phospholipids from soybean. Reconstitution was performed in the presence of cholate which was removed by prolonged dialysis. A similar procedure was used to reconstitute calcium transport with preexisting endogenous microsomal phospholipids (3). The pump prepared by the first procedure has a low efficiency and requires an internal trapping agent for calcium. The second procedure does not allow experimental variation of the phospholipid. Both of these problems have now been overcome with a new procedure of reconstitution (4) which avoids detergents and dialysis. The simplicity and rapidity of the method greatly facilitate the exploration of multiple variations in reconstitution.

In this communication, it will be shown that an energy dependent calcium uptake takes place in reconstituted "instant" vesicles and that the efficiency

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TABLE I

Reconstitution of Calcium Pump Without Detergent

Reconstitution was performed with 9 μmoles of phosphatidylethanolamine and 3 μmoles of phosphatidylcholine or with 12 μmoles of crude soybean phospholipid (last experiment). To the dried phospholipids 0.3 ml of 0.2 M potassium oxalate, pH 7.4 and 300 μg protein of ATPase were added. After 12 minutes of sonication, 20 μl of the mixture was assayed for Ca^{++} uptake for 2 min at 23°.

Assay conditions	nmoles Ca^{++}/min/mg protein
Complete	181
" without ATP-Mg	15
" without ATP	8
" plus 23187	4
" plus 1799 (40 μM)	152
" plus valinomycin (2 μg)	213
" plus valinomycin + nigericin (2 μg)	218
" with crude phospholipids	132

TABLE II

Effect of Time of Sonication on Ca^{++} Uptake and ATPase Activity

In experiment 1 purified phosphatidylethanolamine (9 μmoles) and phosphatidylcholine (3 μmoles) from soybean and 3.2 μmoles of bovine cardiolipin were used for the reconstitution of 300 μg of ATPase in a final volume of 0.3 ml potassium oxalate. In experiment 2, crude soybean phospholipids (17 μmoles) were used. ATPase activity was determined after deproteinization with 5% trichloroacetic acid. The time of sonication was varied as indicated.

Phospholipids	Time of sonication minutes	Ca^{++} uptake μmoles/min/mg	ATPase μmoles/min/mg	ATPase / Ca^{++} uptake
Exp. 1				
Purified	1	0.13	2.0	15
	3	0.31	1.1	3.5
	6	0.42	1.0	2.4
	12	0.41	0.8	1.9
	30	0.32	0.5	1.5
Exp. 2				
Crude soybean	1	0.09	4.0	44
	3	0.18	1.5	8.3
	6	0.29	0.7	2.4
	12	0.21	0.6	2.8
	30	0.10	0.6	6.0

TABLE III

Effect of Phospholipid Composition on Ca^{++} Uptake

Various phospholipids were reconstituted with 300 ug of ATPase in a volume of 0.3 ml in the presence of potassium oxalate. The μmoles phospholipids used are indicated in parenthesis. PC = phosphatidylcholine; PE = phosphatidylethanolamine; C = cardiolipin

	Phospholipids	Ca^{++} uptake nmoles/min/mg
Exp. 1	Crude soybean (12)	190
	PC (12)	11
	PC (6) + PE (6)	40
	PC (9) + PE (3)	40
	PC (3) + PE (9)	140
Exp. 2	Crude (17)	130
	PC (3) + PE (9)	130
	" " + C (0.8)	290
	" " + C (1.6)	400
	" " + C (2.4)	270
Exp. 3	Crude (10)	190
	PC (10)	6
	PE (10)	10
	PC (2) + PE (8)	120
	PC (5) + PE (5)	60
	PC (5) + PE (5) + C (4)	330

and other properties are dependent on the time of sonication and on the phospholipid composition as well as the phospholipid:protein ratio.

MATERIALS AND METHODS

Ca^{++} ATPase from rabbit muscle was prepared according to MacLennan (2). Preparation of phospholipids and the procedure of sonication were as described in the accompanying paper (3). The ionophore 23187 was generously supplied by Dr. R. J. Hosley of Eli Lilly. The reconstitution of vesicles were usually performed in the presence of 0.4 M potassium phosphate buffer, pH 7.4 or 0.2 M potassium oxalate, pH 7.4 except when specified otherwise in the legends to the tables. Ca^{++} uptake was measured as described previously (1), except that 100 μM ^{45}Ca rather than 600 μM were used. ATPase activity was measured under identical conditions (2 min at room temperature) with [^{32}P]ATP as substrate (5).

RESULTS AND DISCUSSION

As shown in Table I the instant vesicles prepared with purified soybean phospholipids catalyzed rapid ATP-dependent uptake of Ca^{++}. Both phosphatidylcholine and phosphatidylethanolamine were required for optimal activity. Ca^{++} uptake was sensitive to ionophores for divalent cations but not to ionophores for monovalent cations. The process was also insensitive to proton ionophores (uncouplers of oxidative phosphorylation).

Table II shows the effect of time of sonication on the rate of Ca^{++} uptake and on ATPase activity of the reconstituted vesicles. It can be seen that transport activity appeared after a few minutes of sonication. With prolonged sonication the ATPase activity decreased while the transport activity remained constant. Thus the Ca^{++} uptake of these vesicles became more efficient, resembling that of sarcoplasmic reticulum vesicles. The rate of Ca^{++} uptake by the new procedure was similar to that obtained by the cholate dialysis procedure (1) when compared side by side with the same preparation of ATPase and phospholipids.

It can be seen from Table III that a suitable mixture of phosphatidylcholine and phosphatidylethanolamine can be substituted for the crude mixture of soybean phospholipids. Phosphatidylcholine alone was consistently inactive, while considerably higher activity was observed with some preparations of phosphatidylethanolamine. Small amounts of contaminating phospholipids noted in thin layer chromatograms may have been responsible for these variations.

In the presence of cardiolipin (which markedly stimulated Ca^{++} uptake) the dependency on ATP was less complete than with phosphatidylcholine plus phosphatidylethanolamine, in spite of the fact that the ionophore 23187 effectively inhibited the Ca^{++} uptake. This observation required further study.

Table IV shows the pronounced effect of the ratio of phospholipid to protein on the rate of Ca^{++} uptake. When about 1.8 μmoles of phospholipid per mg of protein was used, the rate of Ca^{++} uptake was less than 20% of that of vesicles prepared with 18 μmoles phospholipid. With either amount of phospholipid,

TABLE IV

Effect of Phospholipid:Protein Ratio and Oxalate on Ca^{++} Uptake

Reconstitution was carried out as described in the legend of Table II with 300 µg protein of ATPase and crude phospholipids in the presence of 0.3 ml of 0.4 M potassium phosphate or 0.4 M KCl-40 mM Tris-Cl, pH 7.4 (where indicated). In the last experiment, 5 mM potassium oxalate was included in the assay.

Phospholipids µmoles	Phospholipid (µmoles) protein (mg)	Ca^{++} Uptake nmoles/min/mg
11	36.3	193
5.5	18	96
1.8	5.9	63
0.9	2.9	67
0.54	1.8	33
0.54 (with KCl)	1.8	1.6
0.54 " " + oxalate in assay	1.8	21

deletion of phosphate or oxalate during reconstitution eliminated most of the Ca^{++} uptake. However, it can be seen that vesicles with lower phospholipid: protein ratio were stimulated considerably by oxalate added during the assay. Thus they resemble sarcoplasmic reticulum vesicles more than vesicles reconstituted with high phospholipids, which show much less stimulation by external oxalate (data not shown).

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