Short communication

Reconstitution and Partial Purification of $Na^+ - Ca^{2+}$ Exchanger from Crayfish Striated Muscle Plasma Membranes

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$Na^+ - Ca^{2+}$ exchanger reconstituted into proteoliposomes has been further purified from heart sarcolemma (Hale et al. 1984) and synaptosomal membranes (Barzilai et al. 1984). Herein, we report results of reconstitution and partial purification of $Na^+ - Ca^{2+}$ exchanger from crayfish (*Astacus fluviatilis*) striated muscle plasma membranes. This preparation, as compared with rabbit striated muscle plasma membranes (Gilbert and Meissner 1982), shows by one order of magnitude higher $Na^+ - Ca^{2+}$ exchange activity (Ruščák et al. 1987). A further advantage of working with the crayfish membranes, contrary to mammalian preparations, is the stability of $Na^+ - Ca^{2+}$ exchange activity in membranes stored at 0 °C for more than one week.

A fraction of striated muscle plasma membranes enriched in $Na^+ - Ca^{2+}$ exchange activity was prepared according to Ruščák et al. (1987). Two light subfractions with high $Na^+ - Ca^{2+}$ exchange were solubilized in a solution containing sodium cholate 60 mmol.l^{-1}, sodium oxalate 10 mmol.l^{-1}, sodium chloride 1 mol.l^{-1}, Tris-HCl, pH 8, 20 mmol.l^{-1}, asolectin or mixture of bovine brain phospholipids 40 mg/ml, and membrane proteins 1 mg/ml. Brain phospholipids were extracted from bovine brains frozen in liquid nitrogen with a chloroform-methanol-water (70:140:56) mixture, butylhydroxytoluene being used as antioxidant. The organic phase was evaporated under N_2 stream, redissolved in chloroform, and phospholipids were then precipitated with excess of cold aceton.

After 12 h solubilisation the detergent — phospholipids — proteins mixture was centrifuged at 100,000 × *g* for 1 h. The supernatant was dialysed overnight against a 1,000 times larger volume of the solubilisation medium without detergent. At the end of the dialysis, the dialysed mixture was diluted (1:6), with 1 mol.l^{-1} NaCl solution, sonicated at 40 kHz 3 × 15 s and centrifuged 3 h at 240,000 × *g* in a Spinco L 80 ultracentrifuge, rotor SW 40 Ti. The obtained sediment of proteoliposomes was washed with 0.2 mol.l^{-1} NaCl, recentrifuged and stirred in 0.2 mol.l^{-1} NaCl solution, with pH adjusted to 8. $Na^+ - Ca^{2+}$ exchange activity in proteoliposomes was assayed by measuring $^{45}Ca^{2+}$ accu-

mulation in proteoliposomes incubated for 1 min in the presence of 20 μmol . l^{-1} $^{45}Ca^{2+}$ in 1 ml of 0.2 mol . l^{-1} NaCl or KCl at room temperature. After 1 min the cooled mixture was filtered through an 0.5 × 1.5 CM Sephadex C-25 column. The effluent was collected on Whatman GF/C filters. Dried filters were dissolved in SLT 41 (Spolana) scintillation cocktail and the radioactivity was measured in a LKB Rackbeta scintillation counter.

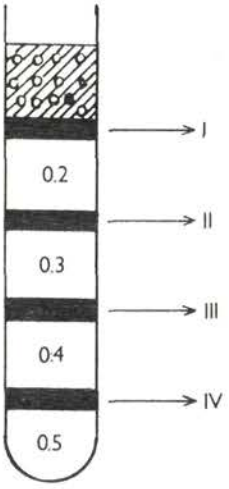

Fig. 1. Schematic diagram representing the separation of proteoliposomes loaded with ^{45}Ca on a sucrose density gradient. Upper part-applied proteoliposomes, symbols I – IV denote the subpopulations of proteoliposomes at the limits of gradients. Numbers inside the diagram indicate sucrose concentrations in mol . l^{-1}.

Proteoliposomes loaded with $^{45}Ca^{2+}$ during $Na^+ - Ca^{2+}$ exchange reaction as described above were further purified by centrifugation on a discontinuous sucrose density gradient (0.2 – 0.3 – 0.4 – 0.5 mol . l^{-1}) at 240,000 × g for 3 h. The subfractions obtained at the limits of the gradients Fig. 1 were sucked off with a syringe and their radioactivity was determined as described above. The protein content of primary proteoliposomes and their subfractions was estimated according to Lowry et al. (1951) after a preceeding repeated 3 × extraction of phospholipids with ether; bovine serum albumin was used as standard. SDS gel electrophoresis of delipidated specimens was carried out according to Laemli (1970).

$Na^+ - Ca^{2+}$ exchange activity taken as the difference in $^{45}Ca^{2+}$ accumulation between KCl and NaCl medium is expressed as nmol $^{45}Ca^{2+}$. mg^{-1} prot . min^{-1}.

A comparison of $Na^+ - Ca^{2+}$ exchange in reconstituted proteoliposomes reveals by 60% higher activity in brain phospholipid preparations. On the contrary, there was a 2.5 times higher passive binding of $^{45}Ca^{2+}$ to asolectin proteoliposomes (Table 1).

It has been stated (Barzilai et al 1984) that the $Na^+ - Ca^{2+}$ exchanger can

Table 1. $Na^+ - Ca^{2+}$ exchange in native plasma membrane vesicles of crayfish striated muscle (I), in asolectin proteoliposomes (II), and brain phospholipid proteoliposomes (III) (nmol $^{45}Ca^{2+}$. mg^{-1} prot . min^{-1}). A — vesicles preloaded in 160 mmol . l^{-1} NaCl transferred into a medium containing 160 mmol . l^{-1} NaCl + 20 μmol . l^{-1} $^{45}Ca^{2+}$. B — vesicles preloaded in 160 mmol . l^{-1} NaCl transferred into a medium containing 160 mmol . l^{-1} KCl + 20 μmol . l^{-1} $^{45}Ca^{2+}$. C — differences between medium B and A, net $Na^+ - Ca^{2+}$ exchange. Mean values of 3 independent experiments. The differences from the mean were between 12 — 16 %.

	A	B	C
I	1.14	7.46	6.32
II	38.4	269.7	231.3
III	15.5	384.6	369.1

only be successfully purified if the exchange activity in proteoliposomes exceeds, at least by one order of magnitude, that in native membranes. This was also confirmed in our experiments. Purification was unsatisfactory in proteoliposomes with only 4 – 5 times higher activities compared to native membranes. If, however, proteoliposomes with an average activity exceeding 35 times that of native membranes were applied on the sucrose gradient (Table 2), the proteoliposomes with 173 times higher $Na^+ - Ca^{2+}$ exchange compared to native membranes were concentrated at the 0.2 – 0.3 sucrose limit. A higher activity than in native membranes was also observed at the 0.3 – 0.4 sucrose limit. In these two proteoliposome subpopulations 58.9% of the total radioactivity applied was found while only 7.6% in the denser regions; their specific $Na^+ - Ca^{2+}$ exchange activity was much lower than that in non-separated proteoliposomes.

Table 2. $^{45}Ca^{2+}$ accumulation in asolectin proteoliposomes separated on sucrose density gradient in nmol . mg^{-1} prot. A — medium $Na_i = Na_o$; B — medium $Na_i - K_o$; C — differences B—A, net $Na^+ - Ca^{2+}$ exchange. Symbols I – IV as in Fig. 1. Total proteins applied on the gradient: 84.5 μg, recovered 67.6 %. Total $Na^+ - Ca^{2+}$ exchange applied on the gradient: 22.5 nmol, recovered 66.5 %.

	A	B	C	% of total $^{45}Ca^{2+}$ uptake	% of total proteins related to C
I	245.1	1341.9	1096.8	32.1	14.2
II	296.8	784.4	478.6	26.8	27.1
III	246.1	425.4	179.3	7.2	19.6
IV	29.8	57.2	27.4	0,4	6.7

Sedimentation of crayfish muscle $Na^+ - Ca^{2+}$ exchange activity reconstituted into proteoliposomes on discontinuous sucrose density gradient resembled that of brain $Na^+ - Ca^{2+}$ exchanger obtained on continuous sucrose density gradient (Brazilai et al. 1984). While the degree of purification from the synaptosomal membranes was 128-fold (Barzilai et al. 1984), and that from heart sarcolemma 80-fold (Hale et al. 1984), the exchanger from crayfish striated muscle plasma membranes was purified 173-fold with regard to native membranes (Table 2). The actual degree of purification was probably even higher considering that some activity (about 85%) of the reconstituted exchanger had been lost during 24 h storage at 0 °C.

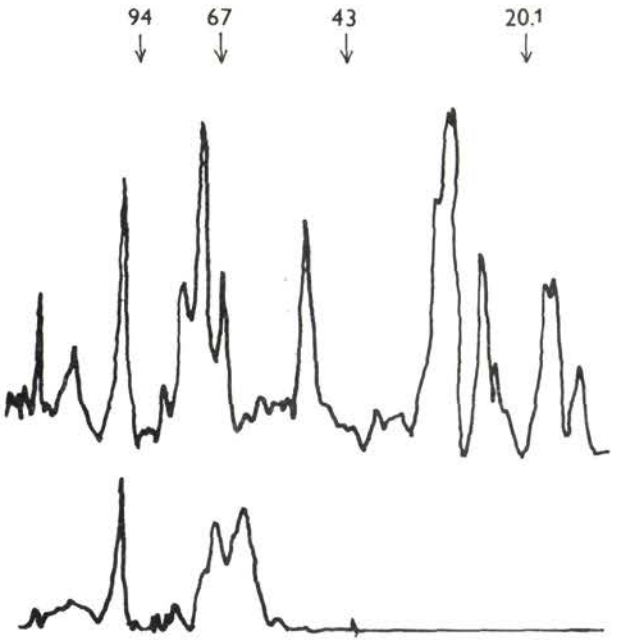

Fig. 2. Densitogram of native membranes (upper trace) and of partially purified proteoliposomes (lower trace — fraction I from Fig. 1). Figures at arrows indicate Mr.

Data concerning M_r of proteins involved in $Na^+ - Ca^{2+}$ exchange mechanism differ considerably. $Na^+ - Ca^{2+}$ exchange activity has been found in heart preparations containing proteins with M_r below 40,000 (Wakabayashi and Goshima 1982) as well as in proteoliposomes enriched in protein with $M_r = 82{,}000$ (Hale et al. 1984). Similar M_r variations could also be observed for brain $Na^+ - Ca^{2+}$ exchange activity (Barzilai et al. 1984, Ruščák et al. 1985). Our partially purified exchanger from crayfish striated muscle showed on SDS

gel electrophoresis an enrichement in a diffuse protein band between M_r 55 — 70,000 (Fig. 2). This value differs from reports of Barzilai et al. (1984) or Hale et al. (1984), who found higher M_r values. In our diffuse protein band two distinct peaks could be observed.

Exact determination of M_r of the $Na^+ - Ca^{2+}$ exchanger requires further separation and functional reconstitution of proteins of the partially purified exchanger.

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