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CARDIAC SARCOLEMMA OF THE HAMSTER

ENRICHMENT OF THE ($Na^+ + K^+$)-ATPase

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Summary

Cardiac sarcolemma was prepared from normal hamsters using gentle homogenization, extraction with 0.6 M KCl and continuous density gradient centrifugation. The final fraction exhibited high ($Na^+ + K^+$)-ATPase activity (24 μmol/mg per h) and contained minimal contamination from mitochondria, myofibrils and lysosomes.

Various attempts have been made to isolate sarcolemma from cardiac muscle [1—7], but none of these preparations have been able to demonstrate more than a two- or three-fold enrichment of the ($Na^+ + K^+$)-ATPase. The major problems involved in the isolation of sarcolemma from cardiac muscle have been the elimination of myofibrillar proteins, mitochondria, lysosomes and sarcoplasmic reticulum. Drastic disruption of cardiac muscle (e.g. 30—60 s homogenization) resulted in fragments of sarcolemmal membranes which were difficult to separate from the above contaminants by differential or sucrose density gradient centrifugation. Tada et al. [2] and Watanabe and Besch [5] homogenized the myocardial tissue for 1 to 2 s; this short period of disruption resulted in morphologically intact sarcolemma which could be harvested with low speed centrifugation. Osmotic shocking of these cellular segments was required before extraction with high salt solution (1.0 M KCl). We have used a longer period of disruption (5 s) in 0.6 M KCl to extract myofibrils [8] and have found that subsequent osmotic shock did not improve the enrichment of ($Na^+ + K^+$)-ATPase. Using this technique, we have accomplished a more than seven-fold enrichment of ($Na^+ + K^+$)-ATPase from our sarcolemmal fraction.

Cardiac sarcolemma was prepared as follows. Hamster hearts were removed from animals that were sacrificed by dislocation of the neck and were im-

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mediately washed with 0.9% NaCl at 4°C. Ventricular tissue was excised and weighted, followed by fine mincing with a razor blade. The minced muscle was placed in 100 ml of 0.6 M KCl/0.25 M sucrose/10 mM imidazole, pH 7.0, (extraction solution), and homogenized with a Potter-Elvehjem homogenizer (ten passes of the pestle). The suspension was disrupted for 5 s at full speed in a Sorvall Omnimixer. The homogenate was centrifuged at 1600 × *g* for 20 min. The pellets were washed twice by resuspension in original volume of extraction solution and recentrifuged at 1600 × *g* for 20 min. The pellet was washed once with 0.25 M sucrose/10 mM imidazole, pH 7.0, to remove excess KCl. This preparation of crude sarcolemma was resuspended in 30% sucrose (w/w)/10 mM imidazole, pH 7.0. A continuous sucrose gradient was formed with equal volumes (18 ml) of 55% sucrose (w/w) and crude sarcolemma in 30% sucrose (w/w); the gradient was centrifuged at 131 000 × *g* for 2 h in an SW27 rotor. Fractions (2.5 ml) were collected from the gradient. The activity of $(Na^+ + K^+)$-ATPase was measured by the difference in the liberation of inorganic phosphate from ATP in the presence and absence of ouabain ($1 \cdot 10^{-4}$ M) [9]. The reaction mixture contained 100—200 μg protein per ml, 100 mM NaCl, 20 mM KCl, 5 mM ATP, 5 mM $MgCl_2$, 0.5 mM EDTA, 20 mM imidazole, pH 7.0 and 20 mM glycylglycine, pH 7.0. Incubation was carried out at 37°C for 10 min. Reaction was stopped by the addition of 0.5 ml of perchloric acid (1.2 M)/8% silicotungstic acid. Inorganic phosphate was measured by the method of Wahler and Wollenberger [10]. Cytochrome oxidase was measured spectrophotometrically by observing the rate of oxidation of ferro-cytochrome *c* at 550 nm at room temperature [11]. *N*-Acetyl-β-glucosaminidase was determined by the method of Woolen et al. [12].

Fig. 1 shows that the crude sarcolemma was separated on the density gradient into two protein peaks. Highly enriched sarcolemma, as denoted by the activity of $(Na^+ + K^+)$-ATPase, was associated with the second peak and was recovered between sucrose concentrations of 38—42% (w/w).

Table I shows the distribution of enzymatic activities measured during

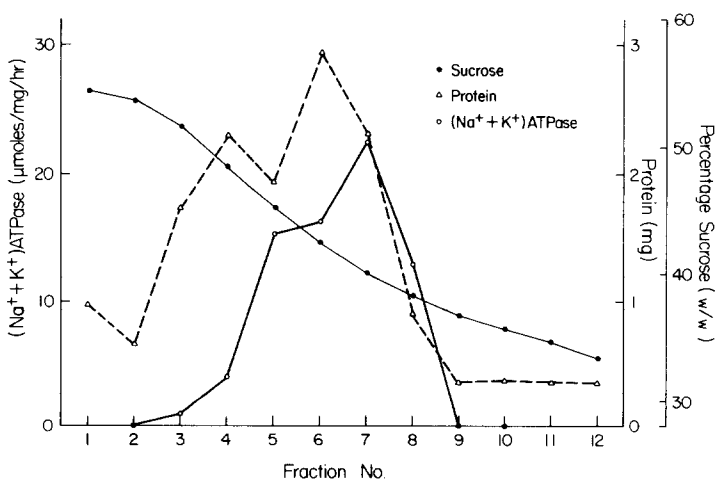

Fig. 1. Sucrose gradient fractionation of cardiac sarcolemma from normal hamsters.

TABLE I

ISOLATION OF CARDIAC SARCOLEMMA FROM HAMSTERS

Specific enzyme activities are measured and expressed as mean ± S.E.M. (μmol/mg per h). Total activity is in μmol/h per g of ventricular muscle.

	Homogenate	Crude sarcolemma	Sarcolemma
Protein			
Yield (mg/g muscle)	159.0 ± 21.1	17.1 ± 1.0	1.42 ± 0.12
% recovery	100	11	0.9
$(Na^+ + K^+)$-ATPase			
Specific activity	3.28 ± 0.33	10.20 ± 1.17	24.04 ± 0.48
Total activity	521.5	174.4	34.1
% recovery	100	33	6.5
Mg^{2+}-ATPase			
Specific activity	12.69 ± 0.75	20.60 ± 2.38	20.42 ± 2.90
Total activity	2017	352	29.1
% recovery	100	22	1.4
Cytochrome oxidase			
Specific activity	35.56 ± 4.93	30.17 ± 6.63	53.46 ± 9.60
Total activity	5654	516	76
% recovery	100	9.1	1.3
N-acetyl-β-glucosaminidase			
Specific activity	0.267 ± 0.006	0.178 ± 0.037	0.057 ± 0.021
Total activity	42.5	3.0	0.081
% recovery	100	7.1	0.2

the isolation of this membrane system. $(Na^+ + K^+)$-ATPase increased more than seven-fold from 3.28 to 24.06 μmol/mg per h. As indicated by the values of cytochrome oxidase, we were unable to eliminate all of the mitochondrial contamination from our preparation; however, the enrichment of cytochrome oxidase was only 1.4-fold. The 1.3% recovery of mitochondria activity was less than that obtained by Hui et al. (2.5%) who were using a similar procedure for the preparation of sarcolemma [7]. Lysosomal contamination was at a minimum of 0.2%.

Our preparation was more enriched in $(Na^+ + K^+)$-ATPase than the cardiac sarcolemma prepared by other investigators (3.6—10.5 μmol/mg per h) [1—7]. This improvement may be due to the exclusion of EGTA [2] and LiBr [1,3] in the extraction solution, since these compounds have been shown to lower the activities of marker enzymes [5,13]. Our slightly longer homogenization time (5 s) compared to that by Tada et al. [2] and Watanabe and Besch [5] (1—2 s) could have improved the extraction of myofibrils by 0.6 M KCl. Increasing the number of washes of crude sarcolemma did not lower the Mg^{2+}-ATPase activity. This may be an indication that the $(Na^+ + K^+)$-ATPase was not detached from the sarcolemma by 0.6 M KCl. Hokin et al. [14] demonstrated that essentially all of the Mg^{2+}-ATPase could be removed on purification of the $(Na^+ + K^+)$-ATPase from brain. Furthermore, the activity of $(Na^+ + K^+)$-ATPase of our preparation (Table I) increased only from 22 to 54% of the total ATPase activity. Matsui and Schwartz [15], in their efforts to purify $(Na^+ + K^+)$-ATPase from calf heart by treatment with deoxycholate and NaI, obtained a fraction containing 96% of the enzyme with only 4% of Mg^{2+}-ATPase. Most probably there is no activation of the $(Na^+ + K^+)$-ATPase by the 0.6 M KCl; we have assayed all the enzymatic activities in the homogenate, as well as in the wash solutions, and we could not recover more than 90% of the total ATPase activities.

This enriched preparation will be useful in studying changes in membrane-

bound enzymes of the sarcolemma of cardiomyopathic hamsters. In addition, we are characterizing the phospholipid-hydrolyzing enzymes of this preparation to study the mechanism of sarcolemmal permeability.

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