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IMPROVED PURIFICATION AND PARTIAL CHARACTERIZATION OF (Na^+, K^+)-ATPase FROM CARDIAC MUSCLE

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SUMMARY

A method is described for purification of (Na^+, K^+)-ATPase which yields approximately 60 mg of enzyme from 800 g of cardiac muscle with specific activities ranging from 340 to 400 μmol inorganic phosphate/mg protein per h (units/mg). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated the presence of a major 94 000 dalton polypeptide and four or five lesser components, one of which was a glycoprotein with an apparent molecular weight of 58 000. The enzyme preparation bound 600–700 pmol of [^{3}H]ouabain/mg protein when incubated in the presence of either Mg^{2+} plus P_i, or Mg^{2+} plus ATP plus Na^+, and incorporated more than 600 pmol ^{32}P/mg protein when incubated with γ-^{32}P-labelled ATP in the presence of Mg^{2+} and Na^+. The preparation is approximately 35% pure.

INTRODUCTION

Purification of (Na^+, K^+)-ATPase (ATP phosphohydrolase, EC 3.6.1.3) from cardiac muscle is of considerable importance because of its probable role as the receptor for digitalis in cardiac muscle. This enzyme has been highly purified from mammalian kidney [1–3], the rectal gland of the spiny dogfish [4] and the eel electroplax organ [5], with specific activities ranging from 800 to 2200 units/mg (μmol P_i/mg/protein per h), and from pig brain [6] with a reported specific activity of 7000 units/mg. In the latter case some difficulty was encountered in determining the protein concentration and the activity was highly labile. Sodium dodecyl sulfate gel electrophoresis of these purified preparations from very different sources indicated a remarkably similar composition, each consisting of a polypeptide with a molecular weight of about 90 000 and a glycoprotein with a molecular weight of 40 000 –60 000. Despite this similarity, enzymes obtained from a variety of sources are not equally sensitive to inhibition by (Na^+, K^+)-ATPase-specific antibodies [7, 8] or by cardiac glycosides [9–12] and this may indicate important, though subtle, structural differences. Consequently, it cannot be assumed that the structure and properties of these enzymes are identical to those of the cardiac enzyme.

Despite the probable role of (Na^+, K^+)-ATPase as the cardiac glycoside receptor in cardiac muscle [10, 11], purification of the enzyme from this source has

lagged far behind that from other tissues. Initial attempts to isolate (Na^+, K^+)-ATPase from cardiac muscle by Repke [13], Schwartz [14] and Auditore and Murray [15] yielded specific activities (ouabain-sensitive) no higher than 16 units/mg. An improved procedure by Matsui and Schwartz [16] and other similar procedures [17–20] yielded specific activities up to 40 units/mg. Shirachi et al. [21] showed that the enzyme could be solubilized from such preparations with Lubrol, but this did not increase the specific activity. Smith et al. [22] recently reported an increase in the specific activity, using the same detergent, to about 75 units/mg. Our preliminary attempts to resolubilize an Na I-treated microsomal preparation with sodium deoxycholate (Pitts et al. [23]) resulted in a substantial increase in specific activity, to about 180 units/mg. Preparations with specific activities of 166, 170, 189 and 188 units/mg were obtained from dog, guinea pig, pig and beef heart, respectively, by this method.

We report here an improved purification method by which specific activities of 400 units/mg or higher may be obtained.

METHODS

Preparation of fragmented membranes

All procedures were carried out at 0–4 °C. The pericardium, endocardium and arteries were removed from the left ventricles of fresh beef hearts. The trimmed muscle (200 g) was chopped into small pieces and blended in a Waring blender for 30 s at low speed with 300 ml 0.25 M sucrose, 1 mM Tris-EDTA (pH 7.0). Sucrose-EDTA (300 ml) was added and the suspension was blended for an additional 45 s at high speed. Sucrose-EDTA (600ml) was added, and the homogenate was filtered through four layers of cheesecloth and centrifuged at 12 000 × g for 10 min (Sorvall GSA rotor). The pellet was resuspended in sucrose-EDTA (400 ml) and blended for 60 s at low speed. The blender was rinsed with 50 ml sucrose-EDTA and the washings were combined. Sodium deoxycholate (Fisher Scientific Co., lot 733392, 40 ml of a 5 % solution in sucrose-EDTA) was added dropwise to the homogenate, which was stirred for 20 min and then centrifuged at 96 000 × g for 25 min (Beckman 35 rotor). The supernatant was recovered by aspiration, diluted to 840 ml with 1 mM EDTA (pH 7.0) and centrifuged again at 96 000 × g for 90 min (Beckman 35 rotor). The pellet was resuspended in 80 ml 1 mM EDTA and stored at −20 °C. Poor sedimentation occurs in subsequent steps if the enzyme is not frozen at this stage, but storage for more than four days should be avoided since this results in substantial loss of activity.

Sodium iodide treatment

The fragmented membrane suspensions obtained from 400 g of heart muscle were pooled and washed to remove excess deoxycholate by dilution with an equal volume of 1 mM EDTA and centrifugation at 40 000 × g for 30 min (Sorvall SS-34 rotor). The pellet was resuspended in a final volume of 120 ml in 1 mM EDTA. A solution (30 ml) of 6 M NaI (containing 15 mM EDTA and 150 mM Tris base, pH about 8.4) was added dropwise over a period of one min, stirred for an additional 5 min, diluted with 240 ml of 1 mM EDTA and centrifuged at 96 000 × g for 25 min (Beckman 35 rotor). The supernatant was carefully removed by aspiration to avoid disturbing the relatively soft pellet and was discarded. The pellet was resuspended in 390 ml of 25 mM imidazole-HCl, 1 mM EDTA (pH 7.0) (imidazole-EDTA) and cen-

trifuged again at 96 000 × g for 20 min. The pellet (NaI-treated enzyme) was resuspended in about 40 ml of the same buffer and after determination of protein by the method of Lowry et al. [24] was adjusted to exactly 10 mg/ml.

Deoxycholate-citrate treatment

The ratio of detergent to protein is very critical and best results are obtained by conducting a preliminary trial as follows. Into six tubes are added 1.1, 1.2, 1.3, 1.4, 1.5 and 1.6 ml of NaI-treated enzyme (10 mg/ml), and imidazole-EDTA is added to bring to a total volume of 1.7 ml. Into each tube, in turn, 1.1 ml of 1.5 M sodium citrate (final concentration = 0.55 M) is added, then 0.2 ml of 5 % sodium deoxycholate, mixing briefly on a Vortex mixer after each addition (the order of additions is important). The suspension is incubated at 0 °C for 10 min and centrifuged at 150 000 × g for 15 min (Beckman 50 rotor). Two ml of each supernatant is transferred to tubes containing 1.4 ml (0.65 vol.) of imidazole-EDTA and centrifuged at 150 000 × g for 20 min. The supernatants are transferred to tubes containing 2.3 ml (0.7 vol.) of imidazole-EDTA and centrifuged again at 150 000 × g for 60 min. The pellets are resuspended in 2 ml imidazole-EDTA, and the specific activities of the enzyme are determined. An example of the results obtained in one such experiment is shown in Fig. 1.

Large-scale preparations are then carried out by scaling up the quantities initially added to the tube that resulted in the highest specific activity. Both Beckman 50 Ti and 35 rotors have been used for this purpose. The enzyme activity declines during centrifugation because of inactivation by deoxycholate and sodium citrate. Consequently, if sufficient time were allowed for complete sedimentation of the enzyme in the 35 rotor, considerable loss of activity would occur and low specific activities would result. The optimum centrifugation times for both rotors were determined by trial

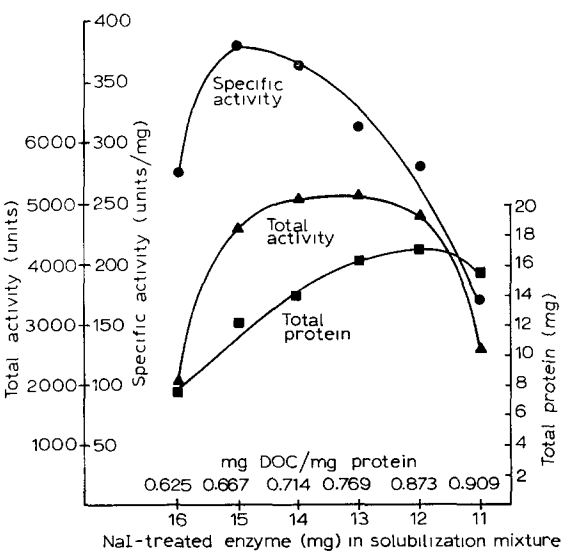
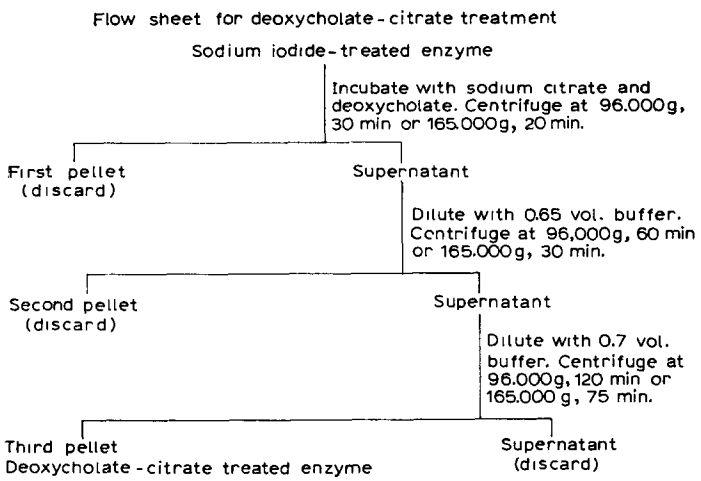


Fig. 1. An example of the results obtained in a typical experiment to determine the optimum ratio of deoxycholate to protein for deoxycholate (DOC) citrate treatment. Details are given in the text.



Scheme 1

and error and are given in the flow diagram above.

When the Beckman 50 Ti rotor is employed, an initial volume of 45 ml is convenient, and the three centrifugation steps are then carried out at 165 000 × g (50 000 rev./min). With the Beckman 35 rotor, an initial volume of 150 ml may be handled, and centrifugation is then carried out at 96 000 × g (35 000 rev./min).

The final pellet (deoxycholate citrate-treated enzyme) is dialyzed overnight against imidazole-EDTA and stored at 0–4 °C. Considerable loss of activity occurs if this preparation (or the NaI-treated enzyme) is frozen. The enzyme loses about 10 % of its activity per month when stored at 0–4 °C.

Determination of (Na^+, K^+)-ATPase activity

Enzyme activity was determined by the spectrophotometric pyruvate kinase/lactic dehydrogenase coupled-enzyme assay perviously described [9], except that 25 mM histidine-HCl (pH 7.2) was employed in place of the Tris buffer. Homogenates and fragmented membrane suspensions were assayed in the presence of 10 mM sodium azide, which considerably reduces the ouabain-insensitive ATPase activity but does not inhibit (Na^+, K^+)-ATPase. One unit of activity is defined as that amount of enzyme which will hydrolyze 1 μmol ATP/h. Consequently, 1 unit/mg is equivalent to 1 μmol P_i/mg protein per h.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

This was carried out as described by Weber and Osborn [25] except that an acrylamide solution containing 16.65 % acrylamide and 0.48 % methylene bisacrylamide was employed to obtain 7.5 % gels. Gels were stained with Coomassie Brilliant Blue (Schwarz-Mann) and scanned at 540 nm or, where indicated, with periodic acid-Schiff stain as described by Glossman and Neville [26] and scanned at 560 nm. Protein samples were extracted with 9 vols of chloroform/methanol (2 : 1, v/v) for 5 min at 37 °C and centrifuged briefly at low speed. The protein residue was washed once in 10 vol of H_2O and taken up in a solution containing 8 % sodium dodecyl sulfate, 10 %

glycerol and 1 % β-mercaptoethanol in 10 mM sodium phosphate (pH 7.1) to a protein concentration of 1 mg/ml. Chloroform/methanol extraction improved resolution of the peaks but did not extract any protein.

RESULTS

Our earlier procedure for purification of the enzyme from cardiac muscle [23] involved deoxycholate solubilization in the presence of NaCl and glycerol precipitation. Gel electrophoresis of the glycerol enzyme in sodium dodecyl sulfate showed that it was not pure (Fig. 2). Subsequently, we found that sodium citrate could be used in place of NaCl to promote solubilization of the sodium iodide-treated enzyme by sodium deoxycholate and that this also resulted in solubilization of much less protein (Fig. 3). The sodium citrate concentration for maximum solubilization was sharply optimal at 0.6 M. (The optimal citrate concentration appears to depend on the ratio of deoxycholate to protein and some variation has been observed in different experiments). Higher concentrations decreased the activity in the supernatant, perhaps due to salting out.

When the enzyme has been solubilized with deoxycholate in sodium citrate, it is necessary to dilute the supernatant in order to sediment the enzyme by high-speed centrifugation. This is partly because the enzyme will not sediment in 0.6 M sodium citrate due to its density, but a reduction in the deoxycholate concentration is also an important factor. Highest recovery and specific activity were obtained in the 165 000 $\times g$ pellet after three-fold dilution of the supernatant (Fig. 4). The maximum specific activity (180 units/mg) was no higher than could be obtained by the glycerol precipitation procedure, but it was of interest that a 1.5-fold dilution permitted the sedimentation of a substantial amount of protein but virtually no activity. This suggested that further dilution and centrifugation of the supernatant should result in a significantly higher specific activity in the final pellet. This proved to be the case, and after a series

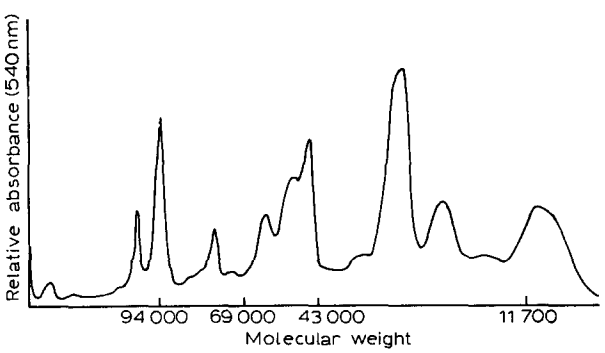


Fig. 2. Sodium dodecyl sulfate gel electrophoresis of glycerol-precipitated (Na^+, K^+)-ATPase from beef heart (specific activity = 134 units/mg). 156 mg of NaI-treated enzyme were incubated for 20 min at 0 °C with 0.4 M NaCl, 0.04 M KCl and 100 mg sodium deoxycholate in a volume of 30 ml in 25 mM imidazole-HCl, 1 mM Tris-EDTA (pH 7.0) and centrifuged at 100 000 $\times g$ (40 000 rev./min, Beckman 50 Ti rotor) for 20 min. The supernatant was diluted with an equal volume of a 20 % solution of glycerol in imidazole-EDTA buffer and centrifuged at 165 000 $\times g$ (50 000 rev./min, Beckman 50 Ti rotor) for 120 min. The pellet was resuspended in imidazole-EDTA and electrophoresed as described in Methods.

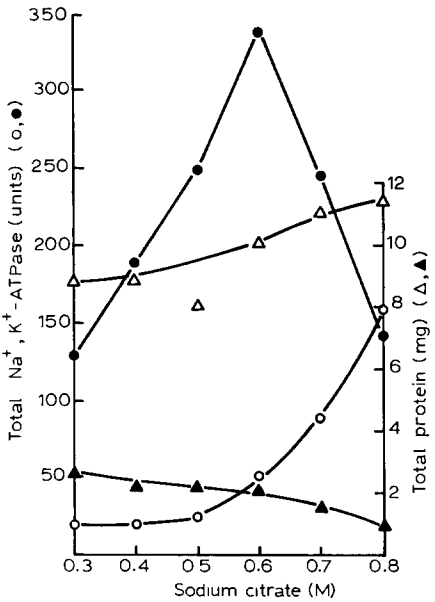


Fig. 3. The effect of sodium citrate on the extent of solubilization of (Na^+, K^+)-ATPase by sodium deoxycholate. Sodium iodide-treated enzyme (15 mg) was incubated for 10 min at 0 °C with the indicated concentration of sodium citrate and 10 mg sodium deoxycholate in a total volume of 3 ml in 25 mM imidazole-HCl, 1 mM EDTA (pH 7.0) and centrifuged at 60 000 × *g* for 30 min. (Na^+, K^+)-ATPase activity in the pellet (○) and supernatant (●), and protein in the pellet (△) and supernatant (▲) are shown in the figure. Direct determination of activity in the supernatant is inaccurate due to inhibition by deoxycholate. To overcome this, the supernatants were diluted to 8 ml with appropriate concentrations of sodium citrate to bring each to a final concentration of 0.2 M and were then centrifuged at 165 000 × *g* for 60 min. The activity in the pellet was taken as a measure of the activity in the original 60 000 × *g* supernatant.

of experiments to determine optimal conditions the procedure outlined in the Methods section was adopted.

The procedure has proved to be quite reproducible, and yields of 50–60 mg of protein (from 800 g of cardiac muscle) with specific activities ranging from 340 to 400 units/mg are generally obtained. It should be noted, however, that the conditions for deoxycholate-citrate treatment are very critical, especially the ratio of deoxycholate to protein, and for this reason it is advisable to conduct a trial experiment as described in the Methods section to determine the optimum ratio. Even with this precaution, preparations have infrequently yielded specific activities as low as 240 units/mg. Occasionally, however, specific activities considerably higher than 400 units/mg have been obtained, and the best preparation had a specific activity of 580 units/mg but lost 20 % of this activity within 24 h.

Table I illustrates the results obtained in a typical preparation. The specific activity was increased more than 120-fold over the activity in the second homogenate with about 9 % recovery of activity. The greatest loss of activity occurred in the preparation of fragmented membranes. Greater activity can be recovered at this stage by increasing the deoxycholate concentration but this greatly increases the amount of ouabain-insensitive activity in the membrane fraction and subsequent sodium iodide

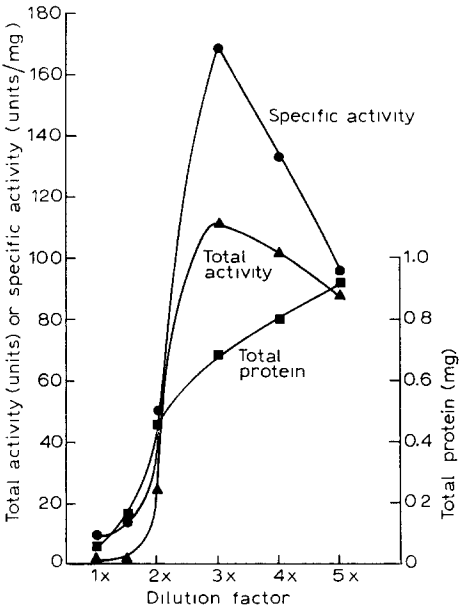


Fig. 4. Effect of dilution of the supernatant enzyme on recovery of activity and protein in the pellet following centrifugation. Sodium iodide-treated enzyme was solubilized with 0.68 mg deoxycholate/mg protein in the presence of 0.6 M sodium citrate and centrifuged at 96 000 × *g* for 30 min. Portions of the supernatant were diluted as indicated in the figure with 25 mM imidazole-HCl, 1 mM EDTA (pH 7.0), and centrifuged at 165 000 × *g* for 60 min. The activity and protein in the pellet are shown as a function of the extent of dilution.

TABLE I

PURIFICATION OF (Na^+, K^+)-ATPase FROM BOVINE HEART*

Fraction	Total protein (mg)	(Na^+, K^+)-ATPase		Total ouabain-insensitive ATPase (units)
		Specific activity (units/mg)	Total activity (units)***	
Homogenate†	10 700	3	31 600	223 000
Fragmented membranes	490	22	10 500	5 890
NaI-treated enzyme	195	41	8 000	520
Discarded fractions**				
1st pellet	151	12	1860	60
2nd pellet	4	71	290	4
Deoxycholate citrate--treated enzyme	7.2	383	2 760	40

* Total activities and protein are from 100 g trimmed left ventricular muscle.

** The discarded fractions are the pellets obtained from the first and second centrifugation steps in the deoxycholate-citrate treatment.

*** One unit is defined as 1 μmol P_i released per h.

† Homogenate obtained after blending the 12 000 × *g* pellet but before the addition of sodium deoxycholate.

treatment is then unable to eliminate it. The ouabain-insensitive ATPase activity in the homogenate was about seven times that of the ouabain-sensitive activity, but 97 % of this was not solubilized by deoxycholate in preparation of the membrane fraction and more than 90 % of the remainder was eliminated by the sodium iodide treatment. The method employed for sodium iodide treatment is a substantial improvement over our earlier procedure [23]. The effectiveness of this treatment in eliminating ouabain-insensitive ATPase activity was improved by increasing the concentration of sodium iodide from 0.6 to 1.2 M, and the loss of (Na^+, K^+)-ATPase activity resulting from this higher concentration was minimized by reducing the time of treatment from 15 to 5 min. The recovery of activity at this stage is 3 to 4-fold higher than obtained with our earlier procedure, and the specific activity is also increased to 40–48 units/mg compared to about 30 with the earlier method. This fraction is still far from pure and contains at least 20 proteins with molecular weights ranging from 12 000 to about 200 000 (electrophoretograms not shown).

Much of the protein in the sodium iodide-treated enzyme is not solubilized by deoxycholate treatment in the presence of sodium citrate and is consequently sedimented in the first centrifugation step. After dilution and centrifugation of the supernatant enzyme, the pellet (which is discarded) contains a considerable proportion of the solubilized protein but very little activity (Table I). The enzyme is then recovered by further dilution and centrifugation and, after washing, has the appearance shown in Fig. 5. The major component, which has a molecular weight of 94 000, together with peak C (a glycoprotein with an apparent molecular weight of 58 000) appear to correspond to the two polypeptides found in highly purified preparations from other sources [1–5]. The other protein peaks correspond to molecular weights of 107 000 (A), 52 000 (D), 48 000 (E), 33 000 (F) and about 12 000 (G).

(Na^+, K^+)-ATPase preparations can be phosphorylated from [γ-^{32}P]ATP and phosphorylation is stimulated by Na^+ and dephosphorylation by K^+ [10, 27]. When a high specific activity cardiac (Na^+, K^+)-ATPase preparation was phosphorylated and subjected to sodium dodecyl sulfate gel electrophoresis, the radioactive label was

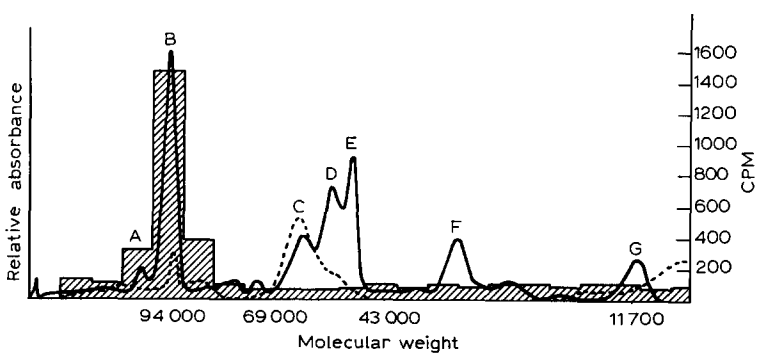


Fig. 5. Sodium dodecyl sulfate gel electrophoresis of deoxycholate citrate-treated enzyme (specific activity = 378 units/mg). (a) 25 μg of enzyme were applied to the gel, electrophoresed as described in the Methods section and stained with Coomassie Blue (solid line). (b) 50 μg of enzyme were applied to the gel, electrophoresed and stained with periodic acid-Schiff reagent as described in the Methods section (broken line). (c) Enzyme was phosphorylated from [γ-^{32}P]ATP in the presence of 100 mM Na^+, electrophoresed, and the gels sliced and counted as described by Lane et al. [2] (hatched bars).

TABLE II

PROPERTIES OF CARDIAC (Na^+, K^+)-ATPase

(Na^+, K^+)-ATPase (units/mg)	342
K^+-*p*-nitrophenylphosphatase (μmol/mg per h)*	59
Phosphorylation (pmol ^{32}P/mg protein)**	
with 20 mM NaCl	639
with 20 mM KCl	18
Ouabain binding (pmol [^3H]ouabain/mg protein)***	677

* Determined as described in ref. 23.

** Method essentially as described in ref. 30.

*** Method as described in the legend to Fig. 6 but incubated with 10^{-6} M [^3H]ouabain in the presence of $Mg^{2+}+P_i$ for 10 min.

found to be associated with the 94 000 dalton peak (Fig. 5). Similar results have been obtained with enzymes from other sources [1, 2, 4, 5] and with crude microsomal preparations from a variety of sources [28]. The levels of Na^+-dependent phosphorylation, ouabain binding and K^+-stimulated *p*-nitrophenyl-phosphatase activity for a preparation with a specific activity of 342 units/mg (Table II) are considerably higher than any previously reported for a cardiac (Na^+, K^+)-ATPase preparation [10].

The rate of [^3H]ouabain binding to the enzyme was fastest in the presence of $Mg^{2+}+P_i$ and maximum binding of 600 pmol/mg was obtained within 4 min (Fig. 6). Binding in the presence of $Mg^{2+}+ATP+Na^+$ was slightly slower, requiring about 8 min for maximum binding, and with $Mg^{2+}+ATP$ or with Mg^{2+} alone, the binding

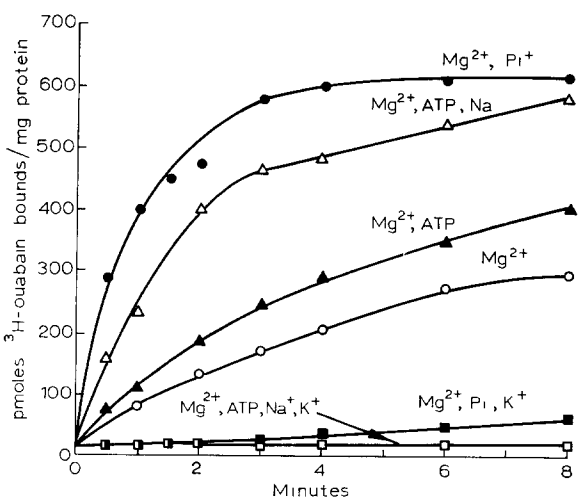


Fig. 6. [^3H]Ouabain binding to cardiac (Na^+, K^+)-ATPase (specific activity 310 units/mg). Deoxycholate-citrate-treated enzyme (0.52 mg) was incubated at 37 °C with $2 \cdot 10^{-7}$ M [^3H]ouabain (120 cpm/pmol), 4 mM $MgCl_2$, 1 mM EDTA and, where indicated, 1 mM ATP, 1 mM P_i, 100 mM NaCl or 10 mM KCl in a final volume of 5 ml in 30 mM histidine-HCl (pH 7.4). At the indicated times, 500-μl aliquots were filtered through a Millipore filter (0.22 μm). The filter was washed once with 5 ml cold deionized water, transferred to a vial containing scintillation fluid and counted. Other details as previously described [31].

process was slower still. Binding in the presence of $Mg^{2+}+P_i$ or $Mg^{+2}+ATP+Na^+$ was inhibited in the presence of 10 mM K^+. These results are qualitatively similar to those obtained with the sodium iodide-treated microsomal preparation [11] but the amount of [^{3}H]ouabain bound per mg protein was about 20-fold higher. The concentration of ouabain used in this experiment ($2 \cdot 10^{-7}$ M) was insufficient to saturate the enzyme. A higher level of binding was obtained at 10^{-6} M ouabain as shown in Table II but at this concentration the rate of binding with $Mg^{2+}+P_i$, or with $Mg^{2+}+ATP+Na^+$ was too fast for a time course to be obtained.

DISCUSSION

Although highly purified (Na^+, K^+)-ATPase preparations have now been obtained from a variety of sources [1–6], the methods employed have not proved directly useful for purification of the enzyme from cardiac muscle. This is largely due to the difficulty in obtaining a pure membrane fraction from cardiac cells because of the high content of organelles and myofibrils and to the relatively low content of the enzyme in the tissue (e.g. one-tenth of that in kidney). Thus, while the (Na^+, K^+)-ATPase activity of sodium iodide-treated microsomes obtained from canine kidney medulla ranges from 250 to 350 units/mg, the activity in the equivalent fraction from bovine heart is no better than 35–45 units/mg. Consequently, application of essentially the same purification procedure (deoxycholate solubilization and glycerol precipitation) to these two fractions yields a much more highly purified enzyme from kidney than from heart. Despite these difficulties, purification of the cardiac enzyme is of considerable importance because of its probable role as the digitalis receptor in cardiac muscle [10, 11, 29].

The procedure described here is only slightly longer than our earlier procedure [23] but the specific activity (about 400 units/mg) is more than twice as high with about the same recovery of activity. The apparent degree of purity indicated by gel electrophoresis is in good agreement with the specific activities. Planimetry of the glycerol-precipitated enzyme shown in Fig. 2 (specific activity = 134 units/mg) indicates that the 94 000 dalton component represents approximately 9 % of the total protein. It is not possible to make a reasonable assessment of the area of the glycoprotein peak in this preparation.

The 94 000 dalton component of the deoxycholate-citrate-treated enzyme (specific activity = 378 units/mg) appears to represent 25 % of the total protein, and the glycoprotein peak (C) may be roughly estimated as 12 % (Fig. 5). If only these two polypeptides are components of the enzyme, it may be approximately 35 % pure. Nakao et al. [6] have suggested that the 94 000 dalton peak is the only component (in a pig brain preparation) and if this is so, our preparation is about 25 % pure.

Although the enzyme preparation obtained by the procedure reported here is not completely pure, it does represent a very considerable improvement over the sodium iodide-treated preparation of Matsui and Schwartz [16] or other similar procedures [17–20] which have been employed in most studies of the cardiac (Na^+, K^+)-ATPase. The procedure is relatively simple, does not require sophisticated equipment and is quite reproducible (the average specific activity of ten preparations was 355 units/mg). The yield of activity (up to 3000 units/100 g of tissue processed) is somewhat higher than obtained by the Matsui and Schwartz procedure [16] despite a 10-

fold higher specific activity. The preparation is able to bind over 600 pmol [^{3}H]ouabain/mg protein, which is the highest reported for a cardiac preparation.

A drawback to the procedure described here is that large amounts of tissue (at least 50 g) must be processed because of the need to conduct a trial experiment to determine the optimum ratio of deoxycholate to protein for the final stage of the procedure. If only small quantities of tissue are available, the deoxycholate solubilization and glycerol precipitation method described in the legend to Fig. 2 is more suitable. This procedure can be carried out with as little as 10 g of trimmed muscle and yields specific activities of 160–180 units/mg.

ADDENDUM

After completion of this manuscript, an abstract (Fed. Proc. 33, 1332, 1974) came to our attention, in which Caragher reported a different procedure for purification of (Na^+, K^+)-ATPase from bovine heart which yielded a specific activity of 740 units/mg. While this is about double that obtained by the method reported here, the yield of enzyme was very low (0.4 mg from 240 g of heart muscle) and the procedure was very much longer.

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