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PURIFICATION AND RECONSTITUTION OF THE $^{32}P_i$-ATP EXCHANGE ACTIVITY OF BOVINE CHROMAFFIN GRANULE MEMBRANE

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Ghosts derived from bovine chromaffin granules have a $^{32}P_i$-ATP exchange activity which is associated with the H^+ pump of that membrane. This activity was low when compared to bacteria, chloroplasts or submitochondrial particles, but had similar properties (K_m for ATP and P_i, ATP/Mg^{2+} ratio, pH profile, inhibition by dicyclohexylcarbodiimide and tributyltin) to the ATPase from above membranes. The $^{32}P_i$-ATP exchange activity was solubilized by cholate/octylglucoside mixtures. The soluble extract was lipid depleted by ammonium sulfate fractionation and partially purified by sucrose gradient centrifugation. The purified preparation was reconstituted with phospholipids by freeze-thawing. The reconstituted vesicles had a $^{32}P_i$-ATP exchange sensitive to dicyclohexylcarbodiimide and trybutyltin and an ATPase with a sensitivity to the inhibitors which varied with the reconstitution conditions. The α- and β-subunits of F_1-ATPase were major components of the preparation.

Introduction

The existence of an ATP-dependent H^+-translocase in the membrane of bovine chromaffin granules, the catecholamine storage vesicles of adrenal medulla, is now well established [1–5]. This ATPase pumps H^+ inside the vesicles, thus generating a proton electrochemical gradient $\Delta\mu_{H^+}$ across the membrane, which in turn is utilized by specific carriers [6–9]. The H^+-translocase is reversible: imposing an artificially generated $\Delta\mu_{H^+}$ on ghosts derived from chromaffin granules results in ATP synthesis and, in addition, a $^{32}P_i$-ATP exchange reaction has been demonstrated [10]. The chromaffin granule H^+-translocase is similar to the mitochondrial ATPase complex, since it is inhibited by DCCD and trisubstituted tin derivatives, but can be distinguished from it since it is resistant to oligomycin [11–14]. Similar activities have been described in other secretory granules [15,16].

In a previous communication [17], we showed that the membrane of chromaffin granules can be solubilized by cholate or deoxycholate and that removal of the detergent from the soluble extract resulted in reconstitution of an active H^+ pump. Evidence supporting this contention is: (i) sensitivity to DCCD or trisubstituted tin derivatives of the reconstituted ATPase; (ii) generation of transmembrane potentials after ATP addition; (iii) synthesis of ATP by an artificially imposed $\Delta\mu_{H^+}$ in reconstituted vesicles; (iiii) presence of a $^{32}P_i$-ATP exchange activity. In the experiments of Ref. 17, the soluble extracts were neither lipid depleted nor purified before reconstitution. This has now been achieved. The $^{32}P_i$-ATP exchange reaction, which, in contrast to ATPase or potential measurements, is related to both the concentration and the

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCCD, *N,N'*-dicyclohexylcarbodiimide.

activity of the H^+ pump, has been used as an assay. We describe the reconstitution of the partially purified $^{32}P_i$-ATP exchange activity.

Materials and Methods

Preparation of chromaffin granule membranes. Bovine chromaffin granules were prepared by centrifugation on discontinuous sucrose gradients [18]. Membranes were derived from the granules by osmotic lysis [17]. Preparations were frozen in liquid nitrogen and were kept at −80°C.

Solubilization and purification of the ATPase complex. All operations were performed at 4°C. Membranes were centrifuged at $100000 \times g_{av}$ for 20 min and incubated at a final concentration of 5 mg protein/ml in 20 mM Tris-HCl buffer (pH 8) containing 50 mM dithiothreitol, 0.4 M ammonium sulfate and 10% methanol for 5 min. Octylglucoside (30 mM) and cholate (0.5%) were then slowly added. The mixture was stirred for 25 min and centrifuged at $1600000 \times g_{av}$ for 60 min. The supernatant was immediately brought to 35% saturation with ammonium sulfate by dropwise addition of saturated neutralized $(NH_4)_2SO_4$. The suspension was centrifuged at $27000 \times g$ for 20 min. The supernatant was brought to 60% saturation of ammonium sulfate. After centrifugation the pellet was resuspended in minimal volume of 20 mM Tris-HCl (pH 8), 0.5 mM dithiotreitol, 0.5 mM EDTA, 1 mM $MgSO_4$ (buffer A). For sucrose gradient centrifugation, cholate (0.2%) was added to the sample (3–5 mg protein) in 0.25 ml buffer A and the mixture was immediately layered on 7–40% (w/v) sucrose gradients in 5 ml of buffer A containing 0.2% cholate and 0.1% asolectin. The gradients were centrifuged at $216000 \times g_{av}$ for 5 h and fractions were collected from the bottom of the tubes.

Preparation of liposomes. Asolectin was dissolved in chloroform, dried under nitrogen and resuspended in buffer A at 40 mg/ml, final concentration. The suspension was sonicated until clarity under nitrogen. In some experiments the solution was centrifuged for 15 min in an Airfuge centrifuge (Beckman) before use.

Reconstitution of the ATPase complex. Samples (100 μg protein in 50 μl) were added to 100 μl of the liposome suspension containing the indicated phospholipid amount. The mixture was rapidly frozen at −70°C and then thawed at 37°C. Where indicated, proteoliposomes were purified by centrifugation of the sample diluted to 2.5 ml with buffer A and layered on 7 ml of 12% sucrose at $160000 \times g$ for 3 h. Pellets were resuspended in the minimal volume of buffer A.

ATPase activity assay. Mg^{2+}-dependent ATPase was assayed as described previously [17].

$^{32}P_i$-ATP exchange activity assay. Samples (100–200 μg protein) were preincubated at 37°C for 20 min in 40 mM Tris-succinate buffer (pH 8). The incubation mixture contained in addition 5 mM ATP, 5 mM $MgSO_4$ and 5 mM $^{32}P_i$ (about $1.5 \cdot 10^6$ cpm) in 0.4 ml final volume. The reaction was initiated by addition of a 20-fold concentrated mixture of the substrates and it was terminated by addition of 50% trichloroacetic acid (50 μl). After centrifugation at $6000 \times g$ for 10 min, a 0.4 ml aliquot of the supernatant was mixed with 0.6 ml of 2.1% ammonium molybdate in 1.08 N H_2SO_4. Unesterified $^{32}P_i$ was extracted three times with 3 ml isobutanol/benzene (1 : 1, v/v) saturated with water. Aliquots (0.5 ml) of the aqueous phase were counted in 5 ml of 50% ethanol by using Cerenkov emission.

Analytical techniques. Proteins were estimated by the method of Lowry et al. [33] with bovine serum albumin as standard, following precipitation in 5% trichloroacetic acid and solution in 2% deoxycholate/3% NaOH. Phospholipid estimation was done by assaying P_i liberated by perchloric acid hydrolysis.

Electrophoresis. Samples from the sucrose gradient were diluted by 1 vol. of 20 mM Tris-HCl buffer (pH 8), desalted by filtration on Ultrogel AcA 202 (IBF, France) and lyophilized. SDS-polyacrylamide gel electrophoresis was performed on 12.5% acrylamide gels [19].

Materials. Bovine adrenals were obtained from the Abattoirs de Mantes (Yvelines, France). Asolectin was from Associated Concentrates (Woodside, NY) and carrier-free $^{32}P_i$ from the Radiochemical Centre (Amersham U.K.)

Results

Characteristics of the $^{32}P_i$-ATP exchange reaction of bovine chromaffin granule membrane

The $^{32}P_i$-ATP exchange reaction was linear with

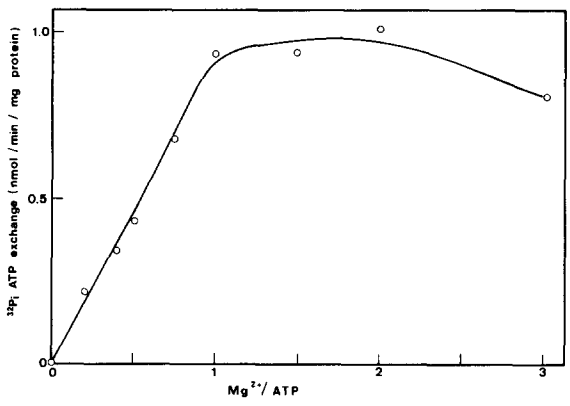

Fig. 1. Effect of Mg^{2+} on $^{32}P_i$-ATP exchange activity. The $^{32}P_i$-ATP exchange activity of membranes (100 μg protein) was assayed as described in Methods, but with 10 mM $^{32}P_i$, 15 mM ATP and variable $MgSO_4$ concentrations. For the zero Mg^{2+}/ATP ratio, EDTA (10 mM) was added to the incubation mixture.

time for more than 45 min and with membrane concentration up to 0.5 mg protein/ml, the highest concentration tested. At 15 mM ATP, a 1:1 Mg^{2+}/ATP ratio was required to obtain maximal reaction rate (Fig. 1). When ATP concentration was varied at constant ATP/Mg^{2+} ratio and P_i concentration, saturation kinetics were observed, characterized by an apparent K_m of 1.9 mM (Fig. 2A). A similar result was obtained when P_i concentration was varied, at constant ATP and Mg^{2+} concentrations (Fig. 2B), from which a K_m of 5.4 mM was derived. The pH dependence of the reac-

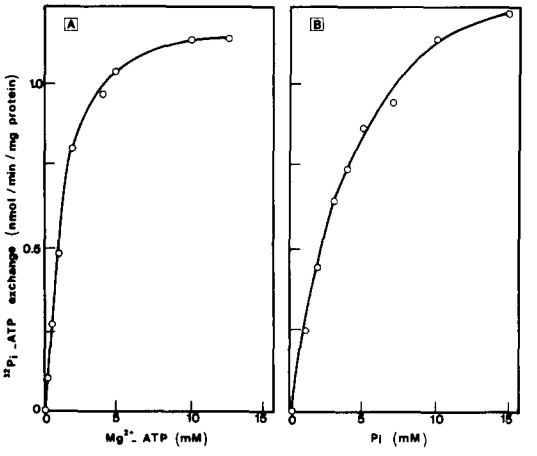

Fig. 2. Effect of substrate concentration on $^{32}P_i$-ATP activity. (A) Effect of ATP concentration. The assay mixture contained 150 μg protein, 10 mM P_i and the indicated ATP/$MgSO_4$ (1:1) concentrations. (B) Effect of P_i. The assay mixture contained 65 μg protein, 15 mM ATP, 15 mM $MgSO_4$ and various amounts of P_i with a constant radioactivity ($1.5 \cdot 10^6$ cpm).

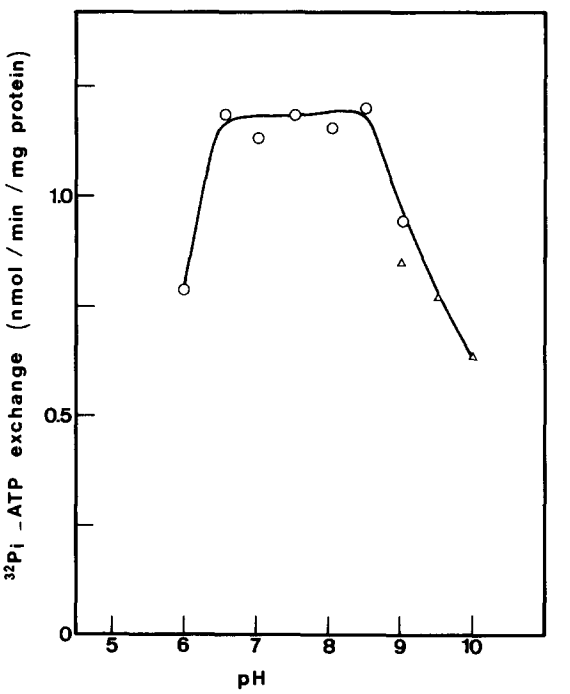

Fig. 3. pH dependence of the $^{32}P_i$-ATP exchange activity. Membranes (160 μg protein) were preincubated in 40 mM buffer (○, Tris-succinate; △, glycine-NaOH) at the indicated pH for 15 min at 37°C. The reaction was initiated by addition of a concentrated mixture of the substrates. Final concentrations were: ATP 15 mM, $MgSO_4$ 15 mM, P_i 10 mM.

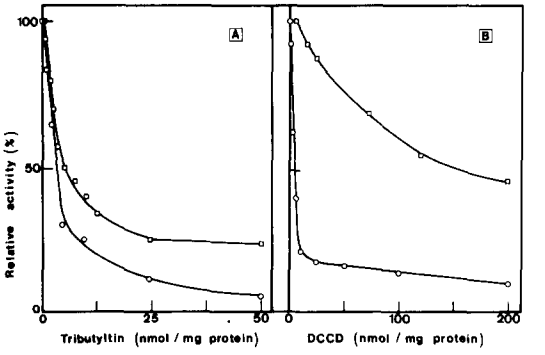

Fig. 4. Effect of tributyltin and DCCD on $^{32}P_i$-ATP exchange and ATPase activities. Membranes were preincubated at 37°C for 20 min with tributyltin chloride (A) or DCCD (B) at the indicated concentrations. $^{32}P_i$-ATP exchange (○) and ATPase (□) activities were assayed with, respectively, 206 and 17 μg membrane protein in 0.2 ml, final volume. Incubations and controls contained 1% ethanol.

tion was studied at optimal substrate concentrations (Fig. 3). In this experiment, the vesicles were first incubated in the absence of the substrates to collapse pH gradients which might result in transient ATP synthesis. The pH profile of the exchange reaction showed a broad optimum between pH 6.5 and 8.5.

The H^+-pump inhibitors DCCD and tributyltin inhibited the $^{32}P_i$-ATP exchange (Fig. 4). It has to be noted that these compounds affected the ATPase and the exchange reaction differently. With both compounds, larger doses were required to inhibit the ATPase than the $^{32}P_i$-ATP exchange reaction. This was clearly seen with DCCD (Fig. 4B) which, at low doses inhibited the exchange without affecting the ATPase.

Solubilization of the exchange activity

Solubilizing conditions allowing optimal lipid depletion and reconstitution of the $^{32}P_i$-ATP exchange activity were investigated. Lipid depletion was achieved by precipitation of the solubilized membrane extract with ammonium sulfate at 50% saturation [20,21]. The ammonium sulfate pellet was suspended in the absence of added cholate; the exchange activity was reconstituted by the addition of sonicated solutions of asolectin followed by freeze thawing of the protein/phospholipid mixture [22]. In a previous work [17], the bile salts cholate and deoxycholate were used to solubilize the chromaffin granule H^+ pump. Cholate proved to be effective in the new procedure, but purification of the membrane extract by sucrose gradient centrifugation after ammonium sulfate precipitation was unsuccessful. The neutral detergent octylglucoside at 30 mM concentration also solubilized chromaffin granule membrane, but the yield of $^{32}P_i$-ATP exchange activity in the reconstituted membrane was about 50% of that obtained with 2% cholate. Mixtures of the two detergents were then tested and the results of an experiment where cholate concentration was varied at constant octylglucoside are presented in Fig. 5. Cholate addition increased slightly the protein of the 0–50% saturation ammonium sulfate pellet and decreased its phospholipid content. At 0.5% cholate, the ammonium sulfate pellet had 26% of the membrane protein and contained 0.18 μmol phospholipid/mg protein. The cholate addition

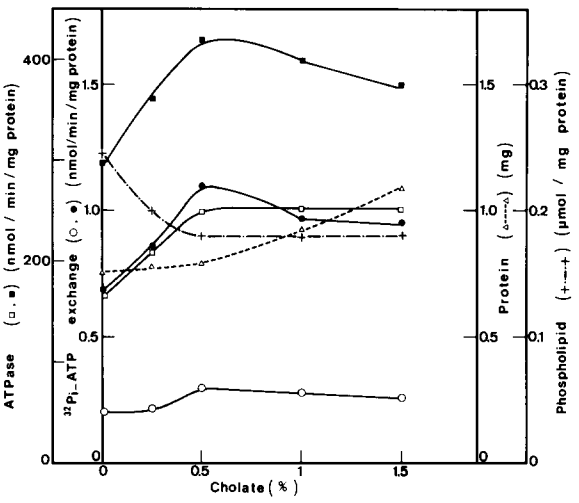

Fig. 5. Solubilization of membranes by 30 mM octylglucoside and various concentrations of cholate. Membranes (3 mg protein) were solubilized as described in Methods, with the indicated cholate concentration. After centrifugation at 160000 × *g*, supernatants were brought to 50% saturation with ammonium sulfate and the resulting pellets were resuspended in buffer A. Aliquots were assayed for $^{32}P_i$-ATP exchange (○), ATPase (□) protein (△) and phospholipids (+). The various fractions were then reconstituted as described in Methods, with 15 μmol phospholipid/mg protein, and assayed for $^{32}P_i$-ATP exchange (●) and ATPase (■).

resulted in an increase in the reconstituted exchange specific activity (3–4-fold) which was maximal at 0.5% cholate (Fig. 5) and a concomitant increase (about 1.5-fold) in the ATPase activity of the reconstituted vesicles. Membranes were therefore routinely solubilized by 30 mM octylglucoside plus 0.5% cholate.

Reconstitution of the $^{32}P_i$-ATP exchange activity

The lipid depletion and reconstitution steps were then optimized. Ammonium sulfate fractionation of the solubilized material indicated that the active material precipitated between 35 and 60% saturation. The 35% saturation ammonium sulfate pellet, which contained $3 \pm 0.4\%$ ($\pm$S.E., $n = 3$) of the total exchange activity, had a specific activity of 0.11 ± 0.03 nmol/min per mg protein and a lipid content of 0.25 μmol/mg protein whereas the 35–60% fraction contained $32 \pm 9\%$ of the activity, with a specific activity of 1.4 ± 0.15 nmol/min per mg protein and a lipid content of 0.09 ± 0.01 μmol/mg protein.

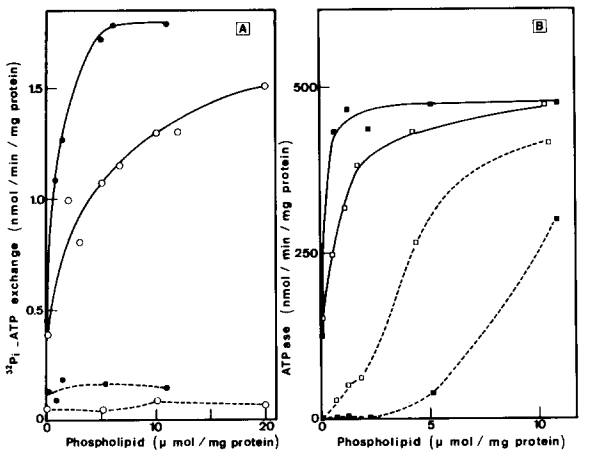

Fig. 6. Effect of phospholipid concentration on the restoration of $^{32}P_i$-ATP exchange (A) and ATPase (B) activities. The 35–60% ammonium sulfate extract was reconstituted with the indicated amount of phospholipids and assayed for $^{32}P_i$-ATP exchange (A) in the absence (○———○) or presence (○— — —○) of 50 μM tributyltin. In this experiment, the inhibitor protein ratio was 0.2 μmol/mg protein. The fractions were then centrifuged on 12% sucrose as described in Methods and the pellets assayed without (●———●) or with (●— — —●) 50 μM tributyltin (0.4–0.5 μmol/mg protein). (B) The same fractions were also assayed for ATPase: without (□———□) or with (□— — —□) 50 μM tributyltin (corresponding to 1 μmol inhibitor/mg protein); after centrifugation, without (■———■) or with (■— — —■) 50 μM tributyltin (2.2–2.7 μmol/mg protein).

Reconstitution of the $^{32}P_i$-ATP exchange activity was dependent upon the concentration of added phospholipids. Asolectin (10–20 μmol/mg protein) induced a 3–4-fold enhancement of the activity. A representative experiment showing activity as a function of added lipids is presented in Fig. 6A. The reconstituted activity was completely inhibited by 5 μM CCCP and by 50 μM DCCD or tributylin (Fig. 6A). It was associated with proteoliposomes, since after centrifugation of reconstituted samples through a 12% sucrose layer ($d = 1.0465$) the pellet contained substantial amounts of the initial activity. Analysis of the effect of asolectin concentration showed that maximal enhancement of the exchange specific activity was observed at lower concentration after the centrifugation step (Fig. 6A). It has to be noted that increasing the asolectin concentration increased continuously the protein content of the pellet.

Exogenous phospholipids affected the reconstitution of the $^{32}P_i$-ATP exchange reaction (Fig. 6A) and that of ATPase (Fig. 6B) in a different way. Asolectin enhanced ATPase activity at concentrations lower than those required for the exchange reaction, the maximal effect occurring at less than 5 μmol phospholipid/mg protein. Moreover, increasing the phospholipid concentration decreased the inhibition by 50 μM DCCD or tributyltin of the reconstituted ATPase, an effect which was not observed on the exchange reaction. Several observations which might be relevant in the explanation of the behavior of the ATPase have been made: (i) the ATPase to which no exogenous phospholipids had been added was sensitive to the drugs only after completion of a freeze thaw cycle; (ii) the ATPase reconstituted at high phospholipid/protein ratio (10 μmol/mg protein) was still inhibited by tributyltin, but a concentration of 500 μM (corresponding to 11.4 μmol drug/mg protein) was required to observe an 80% inhibition (data not shown); (iii) centrifugation of the reconstituted particles through a 12% sucrose layer yielded pellets with an ATPase more sensitive to the drugs (Fig. 6B).

Partial purification of the $^{32}P_i$-ATP exchange activity

After solubilization and ammonium sulfate fractionation, the extract was further purified by centrifugation on a 7–40% (w/v) linear sucrose gradient. The centrifugation was performed in presence of 0.2% cholate to keep the ATPase complex in a soluble form and of 0.1% asolectin to avoid drastic lipid depletion which would impair reconstitution [21]. $^{32}P_i$-ATP exchange activity was measured on fractions individually reconstituted with exogenous phospholipid (Fig. 7). It was inhibited by 5 μM CCCP (90%) and 50 μM tributyltin (80%). Fractions were also assayed for ATPase activity before and after reconstitution. The reconstituted ATPase was moderately stimulated by exogenous phospholipid (Table I) and after reconstitution was sensitive to 50 μM tributyltin or DCCD (85 and 80% inhibition, respectively). It was completely resistant to oligomycin (10 μg/ml). The exchange activity peak was not coincident with that of ATPase, the latter being generally broader than the former.

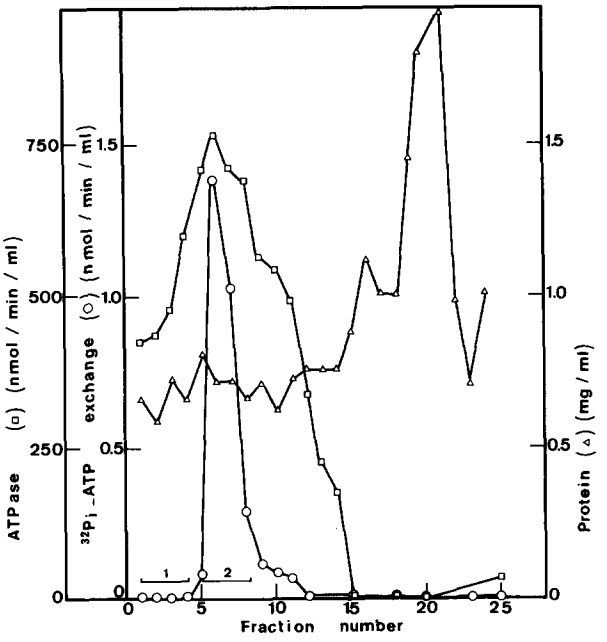

Fig. 7. Centrifugation on sucrose density gradient of the ATPase complex. The 35–65% ammonium sulfate extract (3.7 mg protein) was centrifuged on a 7–40% sucrose concentration gradient as described in Methods. Aliquots (0.1 ml) of the fractions were reconstituted in presence of an equal volume of a 53 mM solution of sonicated phospholipids. They were then assayed for $^{32}P_i$-ATP exchange (○), ATPase (□) and proteins (△).

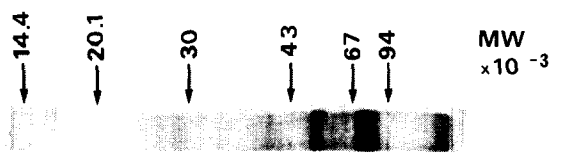

Fig. 8. Analysis of the ATPase complex by SDS-polyacrylamide gel electrophoresis. The sample (20 μg protein of pool 2 from sucrose gradient) was subjected to SDS-polyacrylamide electrophoresis as described in Methods. Standard polypeptides were: phosphorylase *b* (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (20100) and α-lactalbumin (14400).

The results of the purification are shown in Table I. The overall yield of the exchange activity was very low (5–8%) resulting in a very limited increase in specific activity. In contrast, the described procedure resulted in 4-fold purification of the ATPase with a 25–40% yield. The purified preparation has been analyzed by gel electrophoresis on SDS (Fig. 8).

TABLE I

PURIFICATION OF THE ATPase COMPLEX

Fractions were assayed for $^{32}P_i$-ATP exchange and ATPase, with or without addition of phospholipid. In the former case, the samples were reconstituted by freeze-thawing, as described in Fig. 6, with 15 μmol phospholipid/mg protein (step 3) and 7 (step 4). Pools 1 and 2 are indicated on Fig. 7. n.d., not determined.

	Protein (mg)	$^{32}P_i$-ATP exchange		ATPase	
		Specific activity (nmol/min per mg protein)	Total activity (nmol/min)	Specific activity (nmol/min per mg protein)	Total activity nmol/min)
(1) Membranes	38.6	1.3	50.2	205	7913
(2) Solubilized extract	26.3	n.d.	n.d.	218	5733
(3) $(NH_4)_2SO_4$ (35–60%)					
−phospholipid	7.4	0.3	2.2	188	1391
+phospholipid		1.4	10.4	318	2353
(4) Sucrose gradient					
Pool 1	1.2	n.d.	n.d.	334	401
Pool 1+phospholipid		0.0	0.0	722	866
Pool 2	1.3	n.d.	n.d.	716	931
Pool 2+phospholipid		1.9	2.5	973	1265

Discussion

We have previously shown the presence of a $^{32}P_i$-ATP exchange activity in membranes of chromaffin granules prepared by centrifugation through a 1.8 M sucrose layer [10]. By centrifugation of the membranes on 0.45–1.45 M sucrose linear gradients, the exchange activity was shown to be associated with chromaffin granule membrane (assayed by dopamine β-hydroxylase activity and noradrenaline uptake) and not with mitochondria (assayed by monoamine oxidase and cytochrome *c* oxidase). Mitochondrial contamination was estimated to be 5% by measurement, after sucrose centrifugation, of the area of the oligomycin-sensitive ATPase peak, at the level of mitochondrial markers. The $^{32}P_i$-ATP exchange activity of chromaffin granules is low when compared to other systems, about 1.5 vs. 10–20, 50 and 100 nmol/min per mg protein for bacteria [23], chloroplasts [24] and submitochondrial particles [25,26], respectively. As in the other systems, it is sensitive to CCCP, DCCD, trisubstituted tin derivatives and nigericin in presence of K^+ salts [10]. The parameters of the reaction that we have described (K_m for ATP and P_i, ATP/Mg^{2+} ratio, pH profile) do not differ from those reported in the literature for other systems [23–25,27,28]. It has to be noted that $^{32}P_i$-ATP exchange and ATPase reactions differed in some aspects. Thus, the K_m for ATP of the ATPase was 80 μM [7], more than one order of magnitude lower than that of the $^{32}P_i$-ATP exchange. The sensitivity to DCCD and tributyltin of the two reactions were also different (Fig. 4), the H^+ pump being inhibited at lower drugs concentrations. The results of Apps and al. [12] can be interpreted in a similar way, since these authors showed that serotonin uptake (which is dependent upon the H^+ pump activity) was more sensitive to DCCD and tributyltin than ATPase.

Lipid depletion of the solubilized ATPase complex, followed by its reconstitution with total or purified phospholipids, has been reported [20,21]. In one work [20], the effect of uncouplers and DCCD on ATPase activity was taken as an evidence of functional reconstitution. Nevertheless, this approach did not allow purification of the ATPase complex, since the assay was not quantitative. The $^{32}P_i$-ATP exchange reaction is a more quantitative measurement of an active H^+ pump. Moreover, we have systematically assayed our fractions for ATPase and $^{32}P_i$-ATP exchange and we have noted that the latter activity is not always correlated with the presence of a DCCD or tribytyltin-sensitive ATPase. Thus, reconstitution of the 35–60% saturation ammonium sulfate pellet in the absence of added phospholipid (Fig. 6) resulted in a highly sensitive ATPase and a low level of $^{32}P_i$-ATP exchange. It is possible that maximal stimulation of ATPase activity required only a 'boundary' layer of lipid [21] and that more phospholipid had to be present to reconstitute vesicles with a $^{32}P_i$-ATP exchange activity. This hypothesis is supported by the phospholipid concentration dependency of the reconstitution of the two activities (Fig. 6), maximal enhancement of the exchange activity occurring at higher phospholipid concentration [29]. Formation of the putative boundary layer required a freeze-thaw cycle as for incorporation of exogenous phospholipid [22].

We have also obtained vesicles possessing a $^{32}P_i$-ATP exchange activity and a tributyl- and DCCD-resistant ATPase activity: increasing the exogenous phospholipid concentration of the reconstitution mixture increased the $^{32}P_i$-ATP exchange and decreased the effect of inhibitors of the ATPase (Fig. 6). Such a paradoxical effect has been reported [26,30,31]. It might be partly explained by partitioning of the lipophilic drugs in the phospholipid phase, since a centrifugation which removed lipids in excess increased the sensitivity of the ATPase to tributyltin.

The $^{32}P_i$-ATP exchange activity of chromaffin granule membrane proved difficult to purify, since procedures which result in a 5–10-fold increase in the specific activity of the chloroplast [24] or *Escherichia* coli [31] system purified the chromaffin granule activity by a factor of less than two (Table I). The purification yield (5%) was also low, thus raising the possibility that the extraction procedures used might selectively purify the small mitochondrial contaminant. Nevertheless, it has to be pointed out that purification of the mitochondrial ATPase complex by a very similar procedure [26] involving ammonium sulfate fractionation and sucrose gradient centrifugation gave a similar purification factor (1.2–2.1) and yield (6%). The mitochondrial enzyme is thus not more robust

than the granule one and therefore its selective purification appears to be unlikely. The difficulty of the purification of mitochondria and chromaffin granule H^+ pumps is also apparent when ATPase activities are considerd. For the granule ATPase, we report a 4-fold increase in specific activity and a 15% yield (Table I) which have to be compared with a purification factor of 9 and a yield of 10% for mitochondrial ATPase [26]. Purification of the chromaffin granule ATPase complex by affinity chromatography using the dialdehyde derivative obtained by periodate oxidation of ATP was also unsuccessful (data not shown). Since centrifugation of vesicles reconstituted at low phospholipid concentration through a 12% sucrose layer increased the exchange specific activity, several reconstitution cycles might be used as a purification step.

When the purified preparation was analyzed by electrophoresis on SDS-containing polyacrylamide gels multiple bands were apparent. The major components had molecular weights of 75000, 52000 and 50000. The first one is likely to be contaminating dopamine β-hydroxylase, which is the main protein of the membrane. Peptides of 52000 and 50000 are present in ATPases from various origins and in the soluble activity purified from chromaffin granule membrane [11]. They can thus be identified as the α- and β-subunits of F_1-ATPase. The γ-subunit (mol. wt. 28000 [11]) and the DCCD-binding protein (mol. wt. 6600 [32]) are difficult to identify unambiguously on our electrophoretograms.

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