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PURIFICATION AND PROPERTIES OF MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE OF HAMSTER BROWN ADIPOSE TISSUE

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Summary

1. Oligomycin-insensitive ATPase (ATP phosphohydrolase, EC 3.6.1.3) was purified from brown adipose tissue mitochondria. It had a specific activity of 50 units/mg which could be increased up to 85 units/mg by $KHCO_3$. The isolated enzyme represented less than 0.5% of the initial membrane proteins.

2. The enzyme had a molecular weight equal to beef heart ATPase and was composed of five subunits with molecular weights of 56 200, 54 300, 33 500, 13 400 and 9500 respectively.

3. Isolated ATPase was labile while cold and was activated by the divalent cations Mn^{2+}, Mg^{2+}, Co^{2+} and Cd^{2+}. The optimum ATP/Mg^{2+} ratio found was 1.58 and the enzyme had a maximum activity at pH 8.5; the K_m was 220 μM.

4. The ATPase activity was 55% inhibited by aurovertin. The isolated enzyme enhanced the fluorescence of aurovertin, quenched by ATP and Mg^{2+} and enhanced by ADP.

5. Oligomycin sensitivity and cold stability of isolated ATPase was restored by its reconstitution with both brown adipose tissue and beef heart particles depleted of ATPase.

6. The results presented demonstrate that the low ATPase activity of brown adipose tissue mitochondria is due to a reduced content of ATPase.

Introduction

Heat production of brown adipose tissue is the result of intensive oxidation of free fatty acids [1—5]. The catecholamine-induced thermogenesis of brown adipose tissue is produced by the physiological uncoupling of oxidative phosphorylation [6—10]. Therefore for its thermogenic function brown adipose tis-

Abbreviations: SDS, sodium dodecyl sulfate; Tris, tris-(hydroxymethyl) aminomethane; EDTA, ethylenediaminetetraacetic acid.

sue mitochondria require a high oxidative capacity [11—13] without corresponding capacity to synthesize ATP. In 1967 Lindberg et al. [7] demonstrated the low hydrolytic activity of ATPase (ATP phosphohydrolase, EC 3.6.1.3) of brown adipose tissue mitochondria. This was later confirmed by others [14—16] and it was also shown that the ratio of ATPase to cytochrome oxidase in brown adipose tissue mitochondria is 10—20 times lower than in other mammalian mitochondria [15,16]. Similarly it was demonstrated that brown adipose tissue mitochondria possess a low capacity to synthesize ATP [16] and that they exhibit a several-fold increase of State 3 respiration after adding the uncoupler [17]. In addition, the electron microscopy of negatively stained brown adipose mitochondrial membrane completely failed to demonstrate the elementary particles on the inner side of the membrane [7]. The attempt to demonstrate the enhancement of aurovertin fluorescence by brown adipose tissue mitochondria also failed [15]. All these data indicate that in brown adipose tissue mitochondria, the phosphorylating system is not reflected by the oxidative capacity, as in the other mammalian tissues, possibly due either to the low content of ATPase in the mitochondrial membrane, or to a modification of the enzyme itself. The purpose of this study was to purify and characterise mitochondrial ATPase of brown adipose tissue.

The results presented here demonstrate that ATPase can be isolated from brown adipose tissue mitochondria and that the isolated enzyme possesses typical properties of F_1-ATPase. It is shown that the low content of the enzyme rather than its modification is the reason for the low phosphorylating capacity of brown adipose tissue mitochondria.

Materials and Methods

Materials

All chemicals were of the highest purity, commercially available, chiefly from Boehringer Mannheim GmbH, Serva Feinbiochemica GmbH and from Sigma. Other materials and their sources were: Acrylamide-bis (*N,N'*-methylene-bis acrylamide), *N,N,N'N'*-tetramethylenediamine (TEMED) from Koch Light Laboratories Ltd; 2-mercaptoethanol from LOBA-Chemie, Wien, Coomassie brilliant blue (R 250) from BDH Chemicals Ltd. and Sepharose 6B from Pharmacia Fine Chemicals Inc. Aurovertin was a gift from Professor R.B. Beechey.

Isolation of mitochondria, sonicated mitochondrial particles and NaBr-particles

Brown adipose tissue mitochondria were isolated from tissue of Syrian hamsters (120—150 g), acclimatized to cold for 3—4 weeks at 5°C. The isolation was performed according to Hittelman et al. [5] in 0.25 M sucrose, 10 mM Tris/sulfate and 1 mM EDTA, pH 7.4 (medium A) and final resuspension was done in 0.1 M sucrose, 10 mM Tris/sulfate and 1 mM EDTA, pH 7.4 (medium B). Beef heart mitochondria were isolated according to Smith [18].

Submitochondrial particles were prepared by sonic disintegration of a mitochondrial suspension (5 mM Tris/sulfate, pH 7.4, 2—6 mg protein/ml) at 0—4°C using an MSE 100 W ultrasonic disintegrator set at maximum output (3 × 90-s intervals with 30-s pauses). The unbroken mitochondria were sedimented from the sonicate by centrifugation for 10 min at 15 000 × *g*. Submitochondrial

particles were sedimented from the above supernatant by centrifugation for 30 min at 105 000 × *g* and were resuspended in medium A.

ATPase-depleted submitochondrial particles (NaBr particles) were prepared by a 30 min exposure of submitochondrial particles to 3 M NaBr (1 volume of particles and 1 volume of 6 M NaBr). 15 ml of this suspension was overlayered with 2 ml of medium A and centrifuged for 30 min at 105 000 × *g* in a swing-out rotor (3 × 20 ml); NaBr-particles which formed a layer in the sucrose phase were collected and washed twice by medium A (30 min, 105 000 × *g*). Particles were resuspended in medium A at a protein concentration of 8—12 mg/ml and were stored at —20°C.

Isolation of oligomycin insensitive ATPase from hamster brown adipose tissue and from beef heart

Brown adipose tissue ATPase was isolated according to the procedure of Drahota and Houštěk [19] described for isolation of rat liver and beef heart ATPase.

Preparation of Lubrol particles

Isolated mitochondria were resuspended in medium B at a protein concentration of 30—35 mg/ml. From this point all the succeeding steps were carried out at room temperature. A solution of Lubrol WX (19 mg/ml) was added dropwise to the mitochondrial suspension, so that during 2 min a Lubrol concentration 0.15 mg per mg of mitochondrial protein was achieved. The suspension was stirred an additional 3 min, then diluted 5 times by the medium B and centrifuged 30 min at 105 000 × *g*. Lubrol particles were resuspended in the medium B containing 4 mM ATP to give a protein concentration of 20—25 mg/ml.

Solubilisation of the enzyme

Lubrol particles were alkalinized in two steps. The pH was first adjusted by NH_4OH (1 : 5 diluted) at 8.1 and after 15 min at 9.0 After 20 min, the pH of the suspension was adjusted at 8.0 by 1 M Tris/sulfate, pH 7.4, and the suspension was sonicated (MSE 100 W sonic disintegrator) for 10 × 2 min with 1 min pauses. The temperature of the suspension rose during sonication from the initial 25°C to 50°C. The suspension was then cooled to 25°C and centrifuged 30 min at 105 000 × *g*.

Sepharose 6B gel filtration

The solubilized material was applied to the column of Sepharose 6B (2.1 × 76 cm) equilibrated with 0.05 M Tris/sulfate, 1 mM EDTA and 1 mM ATP, pH 7.4. Elution (0.3—0.4 ml/min) was performed with the same buffer. Fractions were collected at 15 min intervals.

Beef heart ATPase was isolated by the chloroform extraction [20]. Beef heart ATPase was further purified by Sepharose 6B gel filtration (conditions as in the above isolation procedure), which increased the specific activity up to 100 units/mg.

Analytical procedures

ATPase activity was assayed by using the release of inorganic phosphate

[21] during 3 min incubation at 30°C in a medium (unless otherwise stated) containing 0.05 M KCl, 0.02 M Tris/sulfate, 3 mM $MgCl_2$ and 5 mM ATP, pH 7.4. ATPase activity was also assayed spectrophotometrically in the presence of the ATP-regenerating system [22] at 30°C using a Beckman Acta III spectrophotometer equipped with an adapter for stirring the sample. 2.7 ml of the medium contained 0.02 M Tris/sulfate, 1.85 mM phospho*enol*pyruvate, 20 μg pyruvate kinase, 10 μg lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27), 0.3 mM NADH (sodium salt), 1.81 mM $MgCl_2$ and 2 mM ATP, pH 8.5. One unit of activity is defined as the amount of enzyme liberating 1 μmol P/min or oxidizing 1 μmol NADH/min under the assay conditions.

Polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate (10% gel) was performed as described by Weber and Osborn [23]. For a typical run of 16 gels, 20 ml of deaerated gel buffer (7.8 g of $NaH_2PO_4 \cdot H_2O$, 38.6 g of $Na_2HPO_4 \cdot 7\ H_2O$ and 2 g of sodium dodecyl sulfate per liter) were mixed with 18 ml of acrylamide solution (22.2 g of acrylamide and 0.6 g of methylene bisacrylamide per 100 ml). After deaeration 2 ml of freshly prepared ammonium persulfate (15 mg/ml) and 60 μl of *N,N,N'N'*-tetramethylene diamine were added. Each tube was filled with 2.2 ml of this solution. Samples were dissociated by 3 min incubation at 100°C in the presence of 1% sodium dodecyl sulfate and 2% mercaptoethanol. Electrophoresis was performed with gel buffer diluted 1 : 1 with water for 30 min at a constant current 3.2 mA per gel followed by 6 h at a constant current 12 mA per gel (25°C). Proteins were stained with Coomassie brilliant blue (R 250) and gels were scanned at 590 nm in a Beckman Acta III spectrophotometer equipped with gel-scanner attachment. For the calibration of gels, the following protein standards were used: bovine serum albumin, ovalbumin, glyceraldehyde-3-phosphate dehydrogenase, creatine phosphokinase, trypsin, myoglobin and cytochrome *c*.

The fluorescence of aurovertin was measured at room temperature using an excitation wave length 366 nm (4 nm slit) and an emission wave length of 480 nm (40 nm slit) in a Perkin-Elmer MPF-2A fluorescence spectrophotometer equipped with a Hitachi chart recorder QPD 33.

Protein was determined by the method of Lowry et al. [24].

Results

Isolation of the enzyme

Several methods were tested to isolate the ATPase of brown adipose tissue mitochondria. The method of Horstman and Racker [25] and that of Catterall and Pedersen [26] solubilized the enzyme only slightly from the membrane. Further purification by ammonium sulfate or protamine sulfate precipitation led to complete inactivation of the enzyme. Furthermore, attempts to extract the enzyme by chloroform [20] were not successful. The solubilized material was impure, yielding only a small percentage of initial activity and its specific activity was low (up to 2 units/mg).

The purification of ATPase of brown adipose tissue was achieved only when the method for isolation of ATPase from Lubrol particles was used [19].

The results of a typical purification experiment are shown in Table I and Fig. 1. Lubrol treatment of mitochondrial membrane increases specific activity three

TABLE I

PURIFICATION OF MITOCHONDRIAL ATPase

Measurements were performed as described in Materials and Methods. ATPase was assayed as the release of inorganic P. For assay, 500 μg of mitochondria, 500 μg of Lubrol particles, 300 μg of sonicate and 10 μg of Sepharose 6B eluate were used.

	Total protein (mg)	ATPase activity		Increase in total ATPase activity (relative to step 1)
		specific (unit/mg)	total (unit)	
Mitochondria	366.2	0.041	15.01	1.00
Lubrol particles	220.0	0.129	28.38	1.89
Sonicate	57.0	0.749	43.09	2.87
Sepharose 6B eluate *	1.23	24.340	29.95	1.99

* ATPase activity eluted at K_{av} 0.364. 25% of applied activity eluated by the void volume was not included in this calculation.

times. Alkalinization and drastic sonication solubilized almost all ATPase activity from Lubrol particles and caused another 1.2—1.5 fold activation. Specific activity of the resultant sonicate was 0.5—0.8 units/mg. The enzyme was separated from other solubilized proteins by Sepharose 6B gel filtration. 70—80% of activity was eluted as a single, symmetrical protein peak (K_{av} = 0.364) and the 20—30%

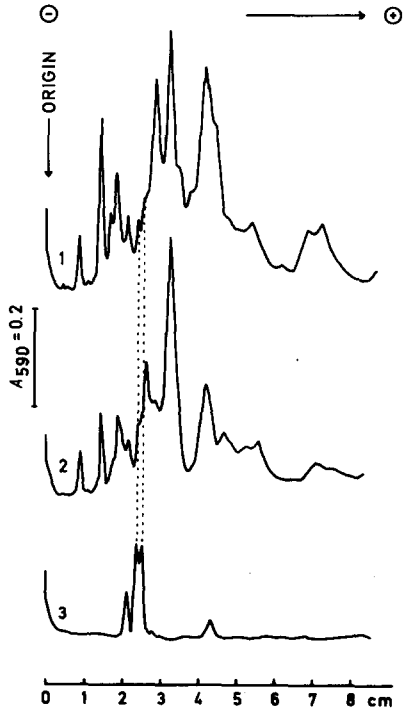


Fig. 1. SDS-polyacrylamide gel electrophoresis of isolated mitochondria, solubilized proteins and of Sepharose 6B eluate. Electrophoresis was performed as described in Materials and Methods. Gels were scanned at a speed of 1.5 cm/min. Trace 1, 25 μg of isolated mitochondria; trace 2, 25 μg of solubilized proteins; trace 3, 1.5 μg of Sepharose 6B eluate (K_{av} = 0.364).

of the activity that remained bound to the membrane fragments, was eluted by the void volume. Total activity of the eluate recovered 95—105% of applied ATPase activity. There was no further activation in this step. Specific activity of the pooled fractions containing isolated enzyme varied between 20—26 units/mg in five experiments.

ATPase activity of the fraction with the highest specific activity was 38—50 units/mg, assayed as a release of inorganic P and 40—55 units/mg assayed spectrophotometrically. Specific activity was increased to 75 units/mg when assayed spectrophotometrically in the presence of 18 mM $KHCO_3$ and to 85 units/mg in the presence of 36 mM $KHCO_3$. The purified enzyme, filtered through Sepharose 6B was eluted as a symmetrical peak at the same elution volume as before and no other proteins were detected in the eluate nor did specific activity markedly increase. Furthermore, sedimentation velocity analysis (Spinco analytical centrifuge, model E, 1.58 mg/ml of 50 mM Tris · Cl, 1 mM EDTA, 1 mM ATP, pH 7.4) showed a symmetrical peak with only a tiny shoulder due to some low molecular weight contamination. It was therefore concluded that the purified enzyme was quite homogenous.

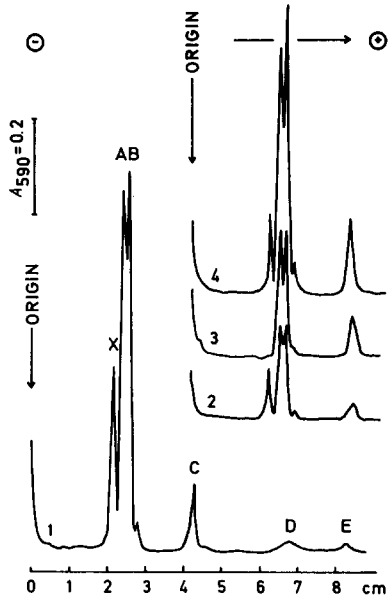
Oligomycin sensitivity and the stability of the isolated enzyme

Purified ATPase of brown adipose tissue was not inhibited by oligomycin. The enzyme was quite stable at 25°C in the presence of 1—2 mM ATP. There was no loss of activity within 36 h and after three days the enzyme still retained more than 50% activity. The purified enzyme was quickly inactivated by cold. When exposed to 0—4°C, the enzyme was 50% inactivated after 30 min, 65% after 2 h and 100% after 5 h.

Molecular weight and subunit composition of the isolated enzyme

With Sepharose 6B gel filtration, brown adipose tissue ATPase was eluted by the same volume (K_{av} = 0.364) as beef heart ATPase. This indicates a molecular weight of about 380 000.

The polypeptide composition of brown adipose tissue ATPase was determined by SDS-polyacrylamide gel electrophoresis on calibrated gels. As demonstrated in Fig. 2, it was composed of six polypeptides X, A, B, C, D and E. The mobilities of polypeptides A, B, C, D and E were similar to the mobilities of subunits of beef heart enzyme. When a mixed sample of brown adipose tissue enzyme and beef heart enzyme was electrophoresed, the corresponding subunits migrated identically in the gel (Fig. 2, tracing 2, 3, 4). Molecular weights of the subunits determined from the migration in gels were calculated as 56 200, 54 300, 33 500, 13 400 and 9500 which values did not differ significantly from values for beef heart enzyme subunits (Table II). The approximate molar ratio of subunits was calculated from the areas of densitometric tracings (gels stained by Coomassie Brilliant Blue) and were close to A_3 B_3 C_1 D_1 E_1 (Table II). The amount of component X relative to other polypeptides varied in Sepharose 6B elution fractions which contained the isolated enzyme. It was highest in the first fraction with ATPase activity and decreased gradually so that in the last fraction X was almost absent. As no relationship between the activity of the enzyme and presence of polypeptide X (molecular weight 61 000) was found, it was assumed that polypeptide X was an impurity.



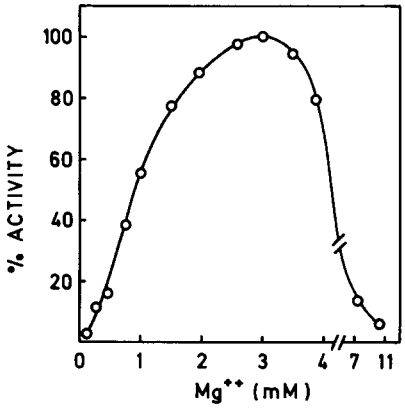


Fig. 2. SDS-polyacrylamide gel electrophoresis of isolated ATPase. Electrophoresis was performed as described in Materials and Methods. Gels were scanned at a speed of 1.5 cm/min. The following amounts of enzyme were used: Trace 1, 4.5 μg of brown adipose tissue ATPase; trace 2, 1.5 μg of brown adipose tissue ATPase; trace 3, 2.0 μg of beef heart ATPase; trace 4, 1.5 μg of brown adipose tissue ATPase +2.0 μg of beef heart ATPase.

Fig. 3. The effect of Mg^{2+} on ATPase activity of the isolated enzyme. ATPase activity of the isolated brown adipose tissue ATPase was assayed as the release of inorganic P, as described in Materials and Methods in the presence of 4.75 mM ATP and a varying concentration of Mg^{2+}. Values are given in % of maximum Mg^{2+} induced activity.

Kinetic properties of the isolated enzyme

Brown adipose tissue ATPase required Mg^{2+} for its activity and activation was dependent on a ATP/Mg^{2+} ratio. In the presence of 4.75 mM ATP the optimum concentration of Mg^{2+} was 3.0 mM which results in the optimum ratio 1.58 (Fig. 3).

TABLE II

SUBUNIT COMPOSITION OF BROWN ADIPOSE TISSUE AND BEEF HEART ATPase

The molecular weights of ATPase subunits were determined by SDS-polyacrylamide gel electrophoresis as described in Materials and Methods. The molar ratio of the subunits was estimated from the relative staining intesities in gels. Areas of the peaks in densitometric traces were divided by corresponding molecular weights of each subunit. Values represent the average of 5 determinations.

Subunit	Brown adipose tissue ATPase		Beef heart ATPase	
	(molecular weight)	(molar ratio)	(molecular weight)	(molar ratio)
A	56 200	35.4%	57 500	32.5%
B	54 300	35.4%	54 300	32.5%
C	33 000	13.4%	33 000	13.0%
D	13 400	8.5%	13 200	11.7%
E	9 500	7.4%	10 000	10.7%
$A_3B_3C_1D_1E_1$	387 400		391 600	

The K_m of the isolated enzyme as determined in the presence of ATP-regenerating system ($ATP/Mg^{2+} = 1.5$) was 220 μM.

The isolated enzyme was also activated by other divalent cations. As shown in Table III, in the presence of 5.0 mM ATP and 3.3 mM metal cation, Mn^{2+}, Co^{2+} and Cd^{2+} were as equally effective as Mg^{2+}.

The activity of the isolated enzyme was dependent on pH (Fig. 4). Maximum activity was found at pH 8.5 and the sigmoidal shape of pH dependence strongly resembled that of beef heart enzyme.

Interaction of the isolated enzyme with aurovertin

The antibiotic, aurovertin, inhibited isolated ATPase of brown adipose tissue, the inhibition being dependent on assay conditions. When the ATPase activity of isolated brown adipose tissue ATPase was measured as the release of inorganic P (the incubation medium contained 20—35 μg of enzyme per ml) 15—20% inhibition was reached with 1.35 μM aurovertin. A higher concentration of aurovertin increased ATPase activity again. When ATPase activity of brown adipose tissue mitochondria was inhibited under these conditions by aurovertin, 50% inhibition was achieved [15].

A pronounced inhibition of the isolated enzyme was achieved when ATPase activity was measured spectrophorometrically in the presence of an ATP-regenerating system, when enzyme concentration in the medium was low (approx. 1.0 μg per ml) and the ratio of aurovertin to the enzyme was high (Fig. 5). Under these conditions, maximum inhibition was 55% with 1.13 μM aurovertin (1 nmol aurovertin/μg of enzyme) and corresponded to maximum inhibition of beef heart enzyme.

The isolated ATPase enhanced the fluorescence of aurovertin as shown in Fig. 6. The enhancement of fluorescence due to 30 μg of brown adipose tissue enzyme (trace B) was the same as fluorescence enhancement obtained with the same amount of beef heart enzyme having the same specific activity (trace A). The subsequent addition of ATP, ADP or Mg^{2+} changed the fluorescence. ATP quenched the fluorescence as did Mg^{2+}, even though the effect of Mg^{2+} was more pronounced and faster (trace A, C). The addition of ADP (trace D) resulted in a small, but significant enhancement of fluorescence.

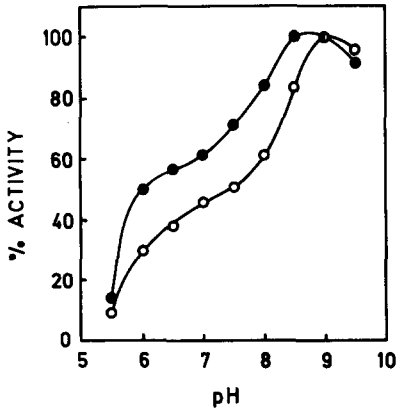
TABLE III

STIMULATION OF ATPase ACTIVITY BY DIVALENT CATIONS

The incubation mixture contained 3.3 mM metal chloride and 5.0 mM ATP and ATPase activity was assayed as the release of inorganic P, as described in Materials and Methods. Values were given as percentages of ATPase activity in the presence of Mg^{2+}.

Cation	Brown adipose tissue ATPase	Beef heart ATPase
Mn^{2+}	131.4	69.0
Mg^{2+}	100.0	100.0
Co^{2+}	96.9	49.2
Cd^{2+}	85.5	28.0
Ca^{2+}	25.6	26.0
Cu^{2+}*	13.6	0.0
None	0.0	0.0

* Sulfate.



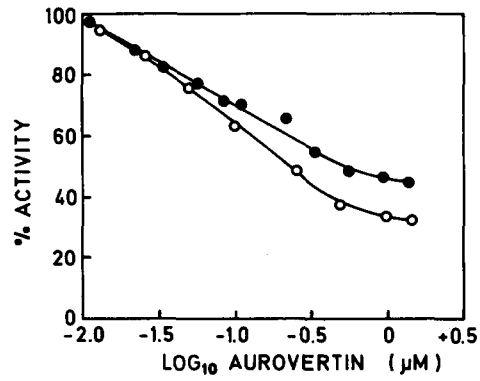


Fig. 4. The effect of pH on ATPase activity of the isolated ATPase. ATPase activity of the isolated brown adipose tissue ATPase (•) and of isolated beef heart ATPase (○) was assayed as the release of inorganic P, as described in Materials and Methods. Instead of Tris/sulfate, the medium contained 50 mM Tris/acetate of the indicated pH. Values are given in % of maximum activity.

Fig. 5. The effect of aurovertin on ATPase activity of the isolated enzyme. ATPase activity was assayed spectrophotometrically as described in Materials and Methods. The reaction was started by 2 mM ATP and 1.33 mM Mg^{2+}. Aurovertin was added prior to ATP. 2.7 ml of media contained 2.7 μg of isolated ATPase of brown adipose tissue (•) or 4.2 μg of isolated ATPase of beef heart (○). Values are given in % of activity in the absence of aurovertin.

Reconstitution of the isolated enzyme with an ATPase-depleted membrane

Oligomycin sensitivity of isolated ATPase of brown adipose tissue was restored by reassociation of the isolated enzyme with a mitochondrial membrane devoid of ATPase activity by NaBr treatment. As shown in Table IV,

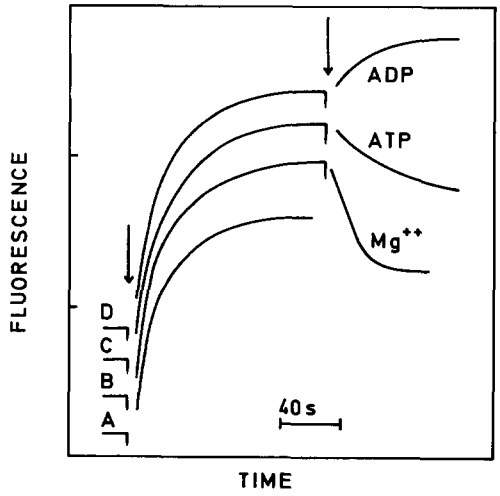


Fig. 6. Interaction of aurovertin with the isolated ATPase. Changes of fluorescence were followed as described in Materials and Methods. 2.1 ml of the media contained 0.25 M sucrose, 50 mM Tris/sulfate, 1 mM EDTA, pH 8.0, and 32 μg of beef heart ATPase of specific activity 29 units/mg (trace A) or 36 μg of brown adipose tissue ATPase of specific activity 30 units/mg (trace B, C, D). At the first arrow 1.8 nmol of aurovertin were added, at the second arrow 2.5 μmol of ADP or 3.0 μmol of ATP or 6.3 μmol of $MgCl_2$ were added.

TABLE IV

RECONSTITUTION OF ISOLATED ATPase WITH NaBr PARTICLES

ATPase and NaBr-particles were isolated as described in Materials and Methods. The above samples were incubated 30 min at 25°C and their ATPase activity was assayed as the release of inorganic P. Oligomycin was present at a concentration of 0.002 mg per sample.

Sample	ATPase activity		Inhibition by oligomycin (%)	Bound ATPase activity (oligomycin sensitive) (units/mg NaBr particles) *
	—Oligomycin (units)	+Oligomycin (units)		
8.4 μg brown adipose tissue ATPase	0.310	0.310	0.0	—
+222 μg brown adipose tissue NaBr particles	0.303	0.278	8.3	0.113
+740 μg brown adipose tissue NaBr particles	0.289	0.228	21.1	0.082
+225 μg beef heart NaBr particles	0.298	0.087	70.8	0.938
+743 μg beef heart NaBr particles	0.304	0.085	72.0	0.295
9.8 μg beef heart ATPase	0.340	0.328	3.5	—
+322 μg brown adipose tissue NaBr particles	0.366	0.321	12.3	0.100
+1072 μg brown adipose tissue NaBr particles	0.344	0.289	16.0	0.040
+215 μg beef heart NaBr particles	0.334	0.135	59.6	0.872
+1084 μg beef heart NaBr particles	0.336	0.128	61.9	0.181

* ATPase bound to particles was calculated as the increase of oligomycin sensitivity due to the presence of NaBr-particles. ATPase activity of single NaBr-particles (lower than 0.01 units/mg) was not taken into this calculation.

oligomycin sensitivity of the isolated enzyme was restored when bound to NaBr particles from brown adipose tissue as well as from beef heart mitochondria. Brown adipose tissue NaBr particles appeared to be less effective. While brown adipose tissue NaBr particles bound 21% of the added activity, beef heart particles bound 70%. Oligomycin sensitivity could be also restored by rat liver NaBr particles (not shown).

When beef heart ATPase was used for reconstitution with the above particles, brown adipose tissue NaBr particles were again less effective (Table IV).

When the maximum capacity of NaBr particles to bind isolated ATPase was determined in similar experiments, it was found that brown adipose tissue NaBr particles could bind 0.09—0.12 units of ATPase activity per mg of particles and beef heart NaBr particles 1.5—2.0 units/mg. These values did not significantly differ from ATPase activity of sonicated submitochondrial particles. Reconstitution of oligomycin sensitivity of brown adipose tissue ATPase by both types of particles also caused cold stability.

Discussion

The experiments reported here describe the purification of ATPase from mitochondria of hamster brown adipose tissue and provide information concerning the properties of the isolated enzyme. Gel filtration and sedimentation velocity analysis revealed homogenity of the enzyme. Only minor contamination by a 61 000 daltons fractions was detected. The molecular weight of brown adipose tissue ATPase calculated from Sepharose 6B gel filtration is in the range of molecular weights reported for mitochondrial ATPases [27—29]. The subunit composition, their molecular weights (A, 56 200; B, 54 300; C, 33 500; D, 13 400 and E, 9500) and their relative molar ratio (close to 3 : 3 : 1 : 1 : 1) correspond to the physical properties of F_1-ATPase preparations [27—29].

The 61 000 daltons contaminant was not connected with particular enzyme activity. Similar contaminants can also be seen in other ATPase preparations, like e.g. in that of Beechey et al. [20], or Johanson and Baltchefsky [30] especially when gels are overloaded. Depending on the molecular weight of polypeptides of mitochondrial membrane in dissociated conditions [31], this component might come from NADH dehydrogenase. Interestingly, brown adipose tissue mitochondrial membrane is very rich in NADH dehydrogenase [13].

The specific activity of isolated ATPase was as high as 50 units/mg, i.e. a 1000-fold increase of specific activity compared to activity of the initial material. This demonstrates the high degree of purification. During the whole procedure, total enzyme activity increased 2.5—3.0 times. This activation would indicate the presence of protein inhibitor. If ATPase from beef heart mitochondria which is rich in protein inhibitor [25,32] is isolated by this method [19] 5—7 fold activation is observed. It is also of interest that the trypsin treatment of beef heart submitochondrial particles activates ATPase activity four times, whereas there is a 1.2-fold activation when brown adipose tissue submitochondrial particles are used [15].

The kinetic properties of isolated ATPase of brown adipose tissue mitochondria resemble the properties of F_1-ATPase preparations of other tissues.

It has similar pH dependence [33], an optimum ATP/Mg^{2+} ratio [34] and the K_m is in the range of values given for F_1 [33,35,36]. It is also activated by anions [37,38]. As other ATPases brown adipose tissue ATPase is inhibited by aurovertin and it enhances the fluorescence of aurovertin [39,40].

The brown adipose tissue enzyme differs from beef heart ATPase only in the relative unspecificity to the stimulatory effect of divalent cations. In comparison with beef heart enzyme, at least three cations Mn^{2+}, Cd^{2+}, and Co^{2+}, were as effective as Mg^{2+} in stimulating an enzyme activity. Such a situation also arises in certain other ATPase preparations, like in thermophilic bacteria *Bacillus stearothermophilus* [29] or rat liver ATPase [38].

The isolated enzyme can be reassociated with membranes depleted of ATPase prepared from brown adipose tissue mitochondria or from beef heart or rat liver mitochondria. The reassociation restores oligomycin sensitivity and induces cold stability. However the binding capacity of the brown adipose tissue mitochondrial membrane is 10—14 times lower when compared with that of beef heart mitochondrial membrane. This could indicate that also oligomycin sensitivity conferring protein and membrane sector of ATPase are reduced in brown adipose tissue mitochondria. It is noteworthy that the maximum reassociated activity (0.12 units/mg) equals the original activity of brown adipose tissue mitochondrial membrane.

On the basis of these findings it can be assumed that ATPase of brown adipose tissue mitochondria possesses features of F_1-ATPase and that it is also similarly bound to the membrane. The discrepancy between the oxidative capacity and phosphorylating capacity of brown adipose tissue mitochondrial membrane, due to low ATPase activity of the membrane, is caused by the reduced content of enzyme in the membrane and not by any qualitative modification of the enzyme.

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