Acta Medica Okayama

Volume 21, *Issue* 4 1967 *Article* 1

AUGUST 1967

Isolation of oligomycin-sensitive adenosine triphosphatase from beef heart mitochondria and analysis of its fine structure

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Isolation of oligomycin-sensitive adenosine triphosphatase from beef heart mitochondria and analysis of its fine structure*

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Abstract

1. An oligomycin -sensitive ATPase was isolated and partially purified from beef heart mitochondria. The specific activity of ATPase sensitive to oligomycin of the fraction was five to eight times that of aged mitochondrial or of DNP-induced mitochondrial ATPase assayed under the same condition. 2. Electron micrographs of the partially purified oligomycin- sensitive ATPase reveal a structure in which headpieces are regularly attached by way of stalks to a thread-like structure derived from a superficial portion of base pieces. 3. A high concentration of the structured material coincided with a high activity of oligomycin-sensitive ATPase. When the headpieces were detached from the structure, the ATPase became insensitive to oligomycin. 4. The fraction of oligomycin -sensitive ATPase was essentially free of membrane structure and was contaminated with a small amount of cytochromes b and Cl but no cyt. a. Cytochrome concentrations of the preparations were indifferent to the activity of oligomycin sensitive ATPase. It follows that ATPase does not require cytochromes or membrane structure for its oligomycin sensitivity. 5. From these results it seems that the factor rendering ATPase sensitive to oligomycin should be contained in the stalks and/or the thread-like portion of basepieces of the structure. The structure is the simplest unit of oligomycinsensitive ATPase as yet obtained. 6. The structure was called "oligomycin-sensitive ATPase particles" (abbreviated as OSA particles). A unit of OSA particles consists of a headpiece attached by a stalk to a portion of base piece.

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*PMID: 4230846 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 21, 147—160 (1967)

ISOLATION OF OLIGOMYCIN-SENSITIVE ADENOSINE TRIPHOSPHATASE FROM BEEF HEART MITOCHONDRIA AND ANALYSES OF ITS FINE STRUCTURE

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Received for publication, June 10, 1967

Regular arrays of uniform particles are seen attached to the surface of the inner membrane of mitochondria[1,2]. The attached particle (head piece), when viewed from its functional aspect, is a part of the elementary particle in the tripartite form composed of head piece, stalk and base piece[2]. Recently it was suggested in RACKER's laboratory[3,4] and in our laboratory[5,8,9] that the isolated head piece is a soluble ATPase (coupling factor F_1), which was purified by PULLMAN *et al.*[6]. The soluble ATPase greatly differs from the membrane-bound ATPase of mitochondria in physico-chemical charactor. In relation to the coupling mechanism of oxidative phosphorylation of mitochondria, the most important difference is that the membrane-bound ATPase is oligomycin-sensitive while the soluble ATPase is not.

Up to the present we have investigated the molecular organization of the mitochondrial membrane by the method of systematic or stepwise microdisection of the mitochondrial membrane by treating with deoxycholate and potassium chloride[7,8,9]. We have isolated and partially purified oligomycin-sensitive ATPase, which was almost free from membrane structure, by the same method. The oligomycin-sensitive ATPase has such a unique structure with the head-pieces combined with stalks to thread-like base pieces. In the following, this structure will be called "oligomycin-sensitive ATPase particles", and will be abbreviated as OSA particles. We also investigated the relationship between oligomycin-sensitive and insensitive ATPase with this fraction, and will discuss what factor may be responsible for the oligomycin-sensitivity.

MATERIALS AND METHODS

Assay of ATPase activity: A buffered substrate solution, consisted of 50 μmoles Tris-chloride, pH 7.4, 6 μmoles ATP, 3 μmoles $MgCl_2$, and water in a final volume of 0.9 ml, was preincubated for 5 min and 0.1 ml of sample was

used. Incubation was conducted at 30° for 10 min. After addition of 1.0 ml of cold 16% perchloric acid the reaction mixture was chilled immediately to 0° and deproteinized by centrifugation. Inorganic phosphate released was estimated by the method of TAKAHASHI[10]. Oligomycin was used at a concentration of 2.5 μg/ml of the reaction medium.

Assays of QH_2-cyt. c reductase, NADH-cyt. c reductase and succinate-cyt. c reductase activities : These enzyme activities were measured as described previously[11].

Preparation of oligomycin-sensitive ATPase : The preparative method for oligomycin-sensitive ATPase bore a resemblance to that of QH_2-cyt. c reductase described in the previous paper[11]. In the following, only an outline of the method is described. For the detailed method of the preparation refer to the previous paper[11].

Beef heart mitochondria were suspended in Tris-sucrose-histidine solution (Tris-chloride, 0.05 M, pH 8.0; sucrose, 0.66 M; histidine, 0.001 M) to a concentration of 23 mg protein per ml. Then potassium deoxycholate (0.3 mg per mg protein) and potassium chloride (72 g per l) were added while stirring. The suspension was centrifuged at 105,000 xg for 30 min, (S_1, R_1). In the following centrifugal fractionation procedure, fractions at each step were labeled as (S_n, R_n); S, supernatant, R, residue, n, fraction number. All subsequent operations were conducted at 0～4°. To S_1, 0.25 volume of chilled distilled water was added with constant stirring and the suspension was centrifuged at 105,000xg for 40 min, (S_2, R_2). The clear supernatant S_2 was dialyzed against 0.01 M Tris-chloride, pH 8.0 for 3 hr. and cloudy dialyzate thus obtained was centrifuged at 105,000xg for 60 min, (S_3, R_3). Oligomycin-sensitive ATPase was prepared not only from R_3 but also from S_3.

Potassium deoxycholate (DOC, 0.5 mg per mg protein) was added to R_3 suspended in Tris-sucrose-histidine solution (10 mg protein per ml). Then an ammonium acetate-fractionation was conducted as described in the previous paper[11]. To R_3 solubilized with DOC, 16.5 ml per cent (v/v) of a 50 per cent saturated ammonium acetate sulution (AmAc) was slowly added with constant stirring. After standing in an ice bath for 15 min, the suspension was centrifuged at 105,000xg for 30 min, (S_4, R_4). To S_4 through S_9, the following ml percentages of 50 % saturated AmAc were added respectively: S_4, 6.5; S_5, 3.2; S_6, 3.2; S_7, 3.5; S_8, 3.5, and S_9, 7.0 to yield R_5 through R_{10} and S_{10}. To S_{10}, 7.0 ml % (v/v) of saturated ammonium acetate were added and the mixture was fractionated, (S_{11}, R_{11}). In these preparation processes, sometimes, pH of the preparation medium dropped to some extent with additions of AmAc, especially in summer season. So pH of the medium must be checked after the

first addition of AmAc and adjusted at 8.0 with 5 N KOH in case of need. The residue at each fraction was suspended in a small volume of Tris-sucrose-histidine solution (T-S-H sol.) and used for the assays of various enzyme activities and electron microscope observation. The remaining samples were stored at $-20°$ and used for the assays of ATPase activity under various conditions.

R_9 and R_{10}, which contained highly active oligomycin-sensitive ATPase and QH_2-cyt. c reductase, were suspended in T-S-H sol. to a concentration of 10 mg protein per ml. Following the addition of neutral potassium cholate (0.4 mg per mg protein) to the sample, cold saturated ammonium sulfate (AS, neutralized) was added to a concentration of 65 ml per 100 ml of protein suspension. After standing in an ice bath for 15 min the cloudy mixture was centrifuged at 105,000 xg for 10 min, ($R_{9,10}$-S_1, $R_{9,10}$-R_1). Then 6.9 ml of AS per 100 ml of supernatant were added to the $R_{9,10}$-S_1 and the mixtnre was centrifuged as before, ($R_{9,10}$-S_2, $R_{9,10}$-R_2). The supernatant was fractionated with 8 ml of AS per 100 ml of the supernatant, ($R_{9,10}$-S_3, $R_{9,10}$-F_3). When a fraction was obtained between 39.4 and 42.0 per cent saturation of AS ($R_{9,10}$-R_2'), this fraction contained more purified oligomycin-sensitive ATPase with less contamination with cytochromes than $R_{9,10}$-R_2.

As previously mentioned oligomycin-sensitive ATPase was also purified from S_3, but the yield of the enzyme was one-third or one-fourth of that obtained from R_3. Contamination of cytochromes in the fraction of oligomycin-sensitive ATPase obtained from S_3 was less than that from R_3. The purification procedure of oligomycin-sensitive ATPase from S_3 was the same as that from R_3 solubilized with DOC. Each fraction obtained from S_3 with ammonium acetate fractionation is designated as (S_3-S_n, S_3-R_n). In this case S_n and R_n correspond with the fraction derived from R_3.

Protein estimation : Protein was estimated by the Biuret method of GORNALL *et al.* [12] and the method of LOWRY *et al.* [13].

Electron microscope observation : Electron microscope observation of each step during the preparation of oligomycin-sensitive ATPase was done by negative staining technique with a JEM 7 electron microseope (Japan Electron Optics Laboratory). Micrographs were taken at an electron optical magnification of 40,000 or 50,000. The detailed method for the preparation of electron microscope specimen was described in the previous paper[11].

RESULTS

ATPase activity of each fraction prepared from beef heart mitochondria by treatment with DOC and KCl, followed by ammonium acetate fractionation, is illustrated in Table 1 and Table 2. A high specific activity of ATPase was

Table 1 ATPase activity in the fractions obtained from beef heart mitochondria by treatment with deoxycholate and potassium chloride

Fractions	Protein (mg)	ATPase activity		
		Specific activity (μmole Pi released /mg protein/min.)	Total activity (μmoles)	% inhibition with oligomycin
Mitochondria	506	0.14	74	90
Mitochondria (DOC)	506	0.11	55.6	86
R_1	113.6	0.12	13.6	88
R_2	23.8	0.14	3.3	90
R_3	97.1	0.49	47.6	97
S_3	92.2	0.21	19.3	96

Table 2 Partial purification of oligomycin-sensitive ATPase from R_3, which was prepared from beef heart mitochondria, by the fractionation with ammonium acetate in the presence of deoxycholate. Precise methods and abbreviations of the fractions are described in the text

Fractions*	Per cent saturation of AmAc	Protein (mg)	ATPase activity		
			Specific activity**	Total activity (μmoles)	% inhibition with oligomycin
R_3		338	0.75	254	92
R_3(DOC)		338	0.61	180	91
R_4	7.1	52.7	0.24	12.7	93
R_5	9.7	19.3	0.46	8.9	82
R_6	11.0	36.5	0.23	8.4	78
R_7	12.3	30.6	0.23	7.1	77
R_8	13.5	46.2	0.22	10.2	85
R_9	14.8	22.2	0.78	17.3	90
R_{10}	17.0	33.8	1.11	37.5	83
R_{11}	29.0	13.4	0.56	7.5	87
S_{11}		10.5	0.72	7.6	48

* These samples had been stored at −20° for a few days before assay of ATPase activity.
** μmoles Pi released per mg protein per min, at 30°.

observed in the R_9 and R_{10}. These values fluctuated to some extent from sample to sample, because ATPase was somewhat labile to DOC treatment. But, in other similar experiments R_9 or R_{10} was the most active preparation of oligomycin-sensitive ATPase in the fractions obtained from R_3 and the specific activity of a fairly good sample was 1.0～1.2 μmoles Pi released per mg of protein per min at 30°; these values were five to eight times the values of aged mitochondrial or DNP-induced mitochondrial ATPase assayed under the same condition. Per

cent inhibition of ATPase activity in R_9 and R_{10} with oligomycin was greater than 80 per cent in all cases. Since R_9 and R_{10} were also rich in QH_2-cytochrome c reductase (as illustrated in the previous paper[11]), it is necessary to separate the ATPase from QH_2-cyt. c reductase. By means of further fractiontion of R_9 and R_{10} with cholate and ammonium sulfate, ATPase was separated from QH_2-cyt. c reductase, but in this fractionation the oligomycin sensitivity of the ATPase dropped to some extent. An example of the data obtained from the fractionation of R_{10} is illustrated in Table 3. Since the concentration of cholate and the temperature during the treatment were very critical, and original sample was a little amount, constant results could not be obtained. In general, ATPase was collected in $R_{9,10}$-R_1 and $R_{9,10}$-R_2 and was scarcely detected in $R_{9,10}$-F_3. In the highest case heretofore obtained, the ATPase activity (R_{10}-R_2') was 3.7 μmoles Pi released per mg protein per min, in which oligomycin-sensitive ATPase activity was 1.3 μmoles Pi released per mg protein per min at pH 7.2, 30°C. As is obvious in this instance, a part of the oligomycin-sensitive ATPase is frequently converted to oligomycin-insensitive ATPase after the treatment with cholate and ammonium sulfate. The oligomycin-insensitive ATPase detectable in these fractions was cold labile and inactivated by freezing-thawing.

Table 3 Further purification of ATPase sensitive to oligomycin from the R_{10} fraction by the fractionation with ammonium sulfate in the presence of potassium cholate

Fractions	Per cent saturation of AmAc	Protein (mg)	ATPase activity		
			Specific activity (μmoles Pi released/ mg protein/min.)	Total activity (μmoles)	% inhibition with oligomycin
R_{10}		45.4	1.25	56.8	90
R_{10}-R_1	39.4	13.9	1.69	23.5	72
R_{10}-R_2	43.4	8.9	0.98	8.7	71
R_{10}-F_3	47.6	14.4	0.28	4.0	71

Consequently, only an oligomycin-sensitive ATPase remained after freezing-thawing, but when freezing-thawings were repeated, it was also gradually inactivated. The $R_{9,10}$-R_1 fraction containing highly active oligomycin-sensitive ATPase was a slightly yellow, turbid solution, and was divided into soluble and insoluble fractions by centrifugation. The $R_{9,10}$-R_2 fraction, also containing the enzyme, exhibits a slightly pink color owning to contamination of QH_2-cyt. c reductase, and such a contamination was minimized in the $R_{9,10}$-R_2' fraction as previously described.

The ATPase activity of each fraction obtained by the fractionation of S_3 with ammonium acetate is illustrated in Table 4. High specific activity of oligomycin-sensitive ATPase was observed in S_3-R_{10} and S_3-R_{11}, which gave slightly

Table 4 Partial purification of oligomycin-sensitive ATPase from S_3, which was prepared from beef heart mitochondria, by the fractionation with ammonium acetate. Precise methods and abbreviations of the fractions are described in the text

Fractions	Per cent saturation of AmAc	Protein (mg)	ATPasc activity		
			Specific activity (μmole Pi released/ mg protein/min.)	Total activity (μmoles)	% inhibition with oligomycin
S_3		530	0.19	98.0	97
S_3-R_4	7.1	262	0.10	25.4	99
S_3-R_5	9.7	46.2	0.21	9.5	100
S_3-R_6	11.0	51.0	0.16	8.2	97
S_3-R_7	12.3	19.0	0.26	4.9	93
S_3-R_8	13.5	7.7	0.35	2.7	87
S_3-R_9	14.8	6.0	0.51	3.0	91
S_3-R_{10}	17.0	6.0	0.96	5.7	86
S_3-R_{11}	29.0	5.3	0.97	5.1	86

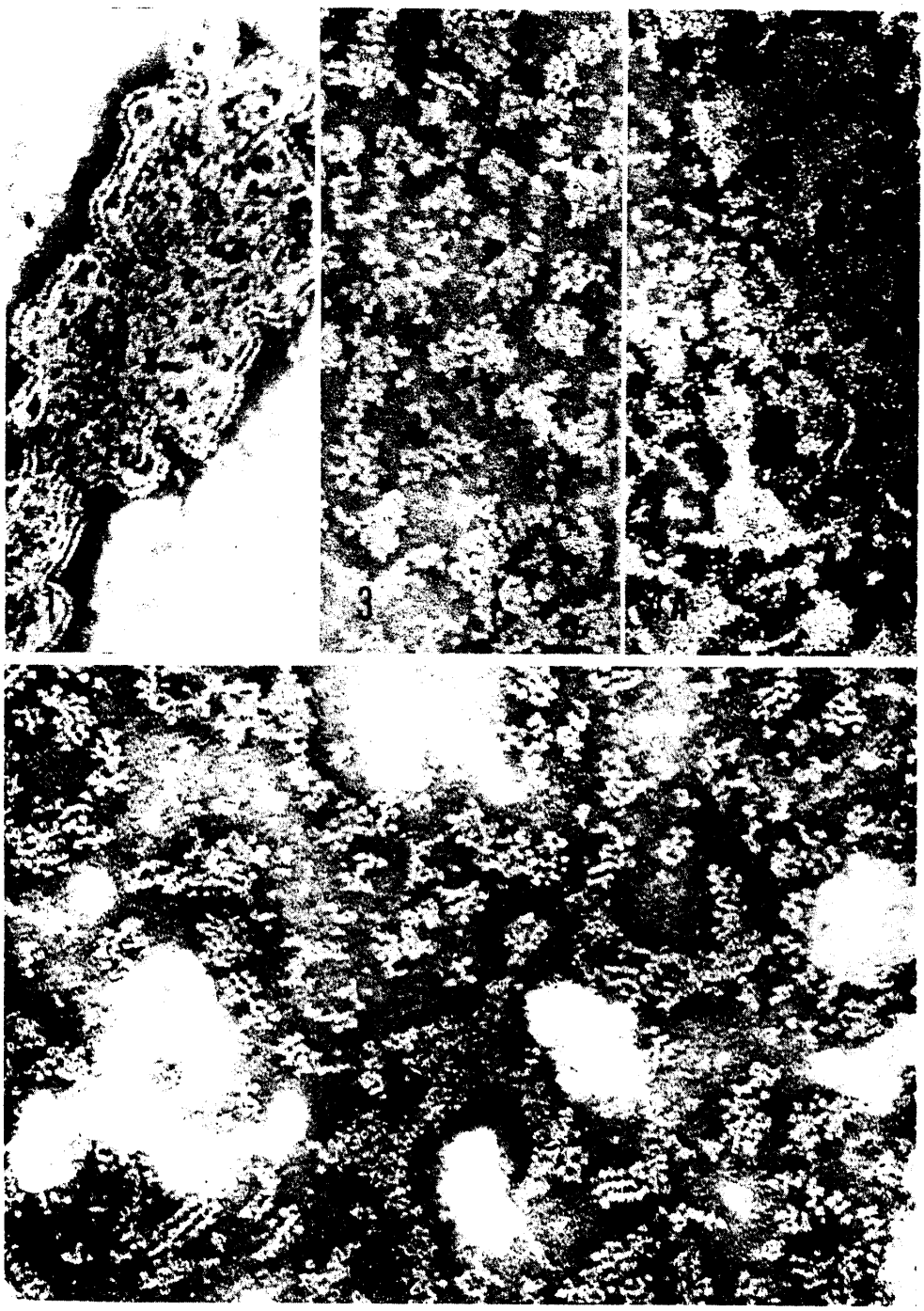
yellow, clear solution. These fractions contained a little contamination of cytochromes b (0.51 mμmole/mg protein) and c_1 (0.10 mμmole/mg protein) but no cyt. a. Even the contamination of cyt. b, which seems to be the highest, is less than 10 %.

Electron microscope observation : Regular arrays of uniform particles (headpieces) are seen on the surface of the inner membrane of mitochondria as illustrated in Fig. 1. When mitochondria were treated with DOC and KCl at an appropriate concentration, a unique structured materal, in which the head pieces combined with thread-like base pieces through stalks, was collected in S_1. For convenience the structure will be called "OSA particles" in following descriptions because it was elucidated in the following experiments that the structured material exhibits a high activity of oligomycin-sensitive ATPase. When S_1 was fractionated by centrifugation after addition of 0.25 vol. of distilled water,

Fig. 1 Inner membrane of mitochondria negatively stained with phosphotungustate (PTA). Regular arrays of head pieces are seen on its surface. ×112,000.

Fig. 2 An electron micrograph of S_2 fraction, negatively stained with PTA, obtained from beef heart mitochondria by treatment with DOC and KCl as described in the text. A unique structure can be observed in which the headpieces are regularly arranged on thread-like base pieces by stalks. One unit of the tripartite structure is called a "unit particle of oligomycin-sensitive ATPase" (OSA particle) for its biochemical nature. ×112,000.

Fig. 3 A OSA particle-rich fraction, negatively stained with PTA, obtained from the R_3 fraction after sonication and fractionation with sucrose density gradient. The OSA particles and aggregates of the particles similar to the headpieces are observed. ×112,000.




4B

the OSA particles were recovered in S_2, and relatively sizeable contaminated or reconstructed membranes were removed in the residue (Fig, 2). The OSA particles have a unique structure in which the headpieces are regularly arranged on both sides of thread-like base pieces linked by stalks. The OSA particles were collected mainly in R_3 obtained from S_2 by the method described previously, but they were also contained in S_3. The R_3 suspended in T-S-H solution gave electron micrographs which showed aggregates, on whose margin a regular array of headpieces was observed, but in which the OSA particles observed in S_1 and S_2 could not be distinguished from one another. When R_3 was sonicated and fractionated on a sucrose density gradient, a fraction in which the OSA particles were relatively concentrated was obtained. The electron micrographs of this fraction dispersed in a low concentration of DOC show the OSA particles and aggregates of the particles which bear a close resemblance to the headpieces (Fig. 3). Similar aggregates were also observed in S_1 and S_2. It may be thought that the particles in the aggregates are mainly QH_2-cyt. c reductase and a small quantity of detached headpieces, but these two different particles could not be distinguished in the figure.

The membranes, which remained in R_3 and could not be dispersed by the treatment with DOC and AmAc, were recovered mostly in R_4, R_5 and R_6, and as a result R_8, R_9 and R_{10} were membrane free in general. The OSA particles and the aggregates of the particles which bore a close resemblance to the head pieces, were mainly recovered in the R_9 and R_{10} (Fig. 4A, 4B). $R_{9,10}$-R_1 and $R_{9,10}$-R_2 obtained from R_9 and R_{10} by the treatment with cholate and AS consisted mainly of small fragments of the OSA particles as well as aggregates whose quantity fluctuated according to the condition of the fractionation. A minute increase in the concentration of cholate and of the temperature during the fractionation caused the headpieces to detach from the OSA particles and as the result the aggregates in $R_{9,10}$-R_1 and $R_{9,10}$-R_2 increased (Fig. 5). Possibly the aggregates consist mainly of the headpieces detached from the OSA particles as a result of the treatment with cholate and AS, and contaminated QH_2-cyt. c reductase. In these fractions ($R_{9,10}$-R_1 and $R_{9,10}$-R_2), a high concentration of the

Fig. 4 A. An electron micrograph of the R_{10} fraction, negatively stained with PTA, obtained from beef heart mitochondria by ammonium acetate fractionation in the presence of deoxycholate. ×112,000
B. An OSA particle-rich part in the photograph of the R_{10} fraction negatively stained with PTA. ×112,000.

Fig. 5 An electron micrograph of the $R_{9,10}$-R_1 fraction, negatively stained with PTA, obtained from the R_9 and R_{10} fraction by ammonium sulfate fractionation in the presence of cholate. This fraction consists mainly of small fragments of the OSA particles as well as aggregates of the particles similar to the head pieces. ×150,000.

Fig. 6 An electron micrograph of the S_3-R_{10} fraction negatively stained with PTA. In this photograph the OSA particles are arranged in ring-like structure. ×130,000.

OSA particles corresponded to a high specific activity of the oligomycin-sensitive ATPase and a high concentration of the aggregates of particles similar to the headpieces corresponded to a high specific activity of oligomycin-insensitive ATPase or increased contamination of QH_2-cyt. c reductase.

The fundamental structure of the OSA particles observed in $R_{9,10}$-R_1 and $R_{9,10}$-R_2 is identical with that observed in S_2, R_3, R_9 and R_{10}; namely, the OSA particles have a structure in which headpieces are arranged on both sides of thread-like base pieces by stalks. But the OSA particles observed in $R_{9,10}$-R_1 and $R_{9,10}$-R_2 differ somewhat from that observed in S_2, R_3 etc. in that the base pieces of the former are shorter than that of the latter and headpieces frequently are missing here and there from the base pieces.

Fractions S_3-R_{10} and S_3-R_{11} consist mostly of the OSA particles, whose structure is somewhat unusual in that the headpieces are regularly arranged on the outside of a ring-like structure of base pieces, linked by stalks, in which the base pieces frequently have a thickened appearance. The thickening is due to lipids once solubilized with DOC and KCl from mitochondria which adhere to the inner side of the base pieces (Fig. 6). Sometimes the lipid micelles cover the entire inside of the ring as if covering it with the film of soap. Interactions between OSA particles and lipid will be reported in the following paper[14]. The OSA particles arranged in rows, was also observed in some place of the same photograph of the S_3-R_{10}.

OSA particles are easily stripped off from the inner membrane of mitochondria by the treatment with a low concentration of potassium deoxycholate (0.1 mg/mg protein) and potassium chloride (72 g/l)[11]. These OSA particles are arranged both in rows as observed in the R_{10}-R_1 fraction and circularly as observed in the S_3-R_{10} fraction. The residual submitochondrial membrane (brown membrane), which is depleted of OSA particles from the inner membrane of mitochondria, contains whole electron transfer components with only exception of cyt. c.

DISCUSSION

As mentioned above, there are many reports dealing with the actual biochemical function of the headpieces of the mitochondrial elementary particle, but up to the present time a final conclusion has not yet been obtained[3,4,15,16,17]. In these reports those of RACKER's laboratory concerning coupling factor F_1 (soluble ATPase) are in noteworthy[3,6]. They suggest that the headpiece is identical with coupling factor F_1. In our laboratory KOSHIBA *et al*[5]. also isolated the head pieces from beef heart mitochondria by the method of a two-step sonication and purified them by a modification of the method of PULLMAN *et al*[6]. It was confirmed that the purified headpieces exhibit a high ATPase activity insensitive to oligomycin[8,9,23]. There still remain some questions as to whether or not all of

the headpieces are ATPases, or whether or not all of the mitochondrial ATPase is head piece only. It is nevertheless doubtless that the headpieces contain oligomycin-insensitive ATPase. Our experimental results described above also indicate the same fact.

On the other hand, there is little information about the biochemical nature of the stalk and base piece.. During the purification process of QH_2-cyt. c reductase we obtained a fraction exhibiting a high activity of ATPase sensitive to oligomycin. In this paper we studied the purification and fine structure of oligomycin-sensitive ATPase. Oligomycin-sensitive ATPase was purified in $R_{9,10}$-R_1 and $R_{9.10}$-R_2', or S_8-R_{10} and S_8-R_{11}. Electron micrographs of the fraction negatively stained with PTA exhibit particles with such a unique structure in high proportion as schematically illustrated in Fig. 7. When the headpieces are

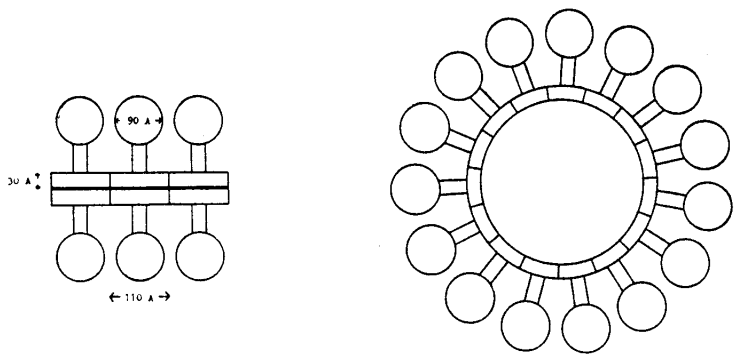


Fig. 7 Diagrammatic representation of the repeating unit particles of oligomycin-sensitive ATPase (OSA particles) observed in R_{10}-R_1 (A) and S_3-R_{10} (B). The OSA particles are arrange ain rows in R_{10}-R_1 and circularly in S_3-R_{10}.

detached from such particles by treatment with cholate and AS, the mixture exhibits ATPase activity insensitive to oligomycin. When mitochondrial phospholipids are added to the mixture, ATPase activity sensitive to oligomycin is restored, as to be reported in a following paper[14].

These data indicate that the ATPase associated with a structure in which the headpieces are combined with threadlike base pieces by stalks is sensitive to oligomycin, but when the headpieces are detached from the such structure the ATPase becomes insensitive to oligomycin. From these results it is clear that the structure of the OSA particles contains a factor giving oligomycin sensitivity to headpieces and the factor is probably either the stalk and/or a portion of the base piece seen in the structure. And it is also suggested that neither cytochromes nor membrane structure is necessary for oligomycin sensitivity of ATPase.

Recently BYINGTON *et al*[18]. reported preparation and properties of an oligo-

mycin-sensitive ATPase from mitochondria. In the abstract they reported that the ATPase was purified 4- to 5-fold and essentially free of electron transport components. No comment concerning the fine structure of oligomycin-sensitive ATPase can be obtained from their abstract.

Factors rendering the ATPase activity of headpieces sensitive to oligomycin, F_0 and CF_0, have been isolated in RACKER's laboratory[19,20,21]. F_0 is a crude mitochondrial fraction still containing electron transfer components and CF_0, which was isolated from T-U particles by fractionation with ammonium sulfate in the presence of 2% cholate, is a large membrane fragment[22]. The smallest unit of oligomycin-sensitive ATPase cannot be identified in these preparations, because both of them are large membrane fragments and the smallest unit of them is not clear. Because the preparation of CF_0 and F_0 is conducted without any intimate correlation to headpiece and respiratory enzymes, it is not clear how the factor relates to headpieces and how it is organized in the mitochondrial membrane.

Our preparation of oligomycin-sensitive ATPase has the simplest structure known thus far and the smallest unit of oligomycin-sensitive ATPase (OSA particle) can probably be indicated. A structural correlation between oligomycin-sensitive ATPase and insensitive ATPase can also be clarified from the fraction. It may be said that the OSA particies localize in the utmost matrix side of the inner membrane of mitochondria with close relation to the electron transfer components and are easily stripped off from the inner membrane of mitochondria by the treatment with a low concentration of DOC and KCl without destruction of the membrane structure. Further study is now in progress concerning the structural correlation between oligomycin-sensitive ATPase and electron transfer components, and the relationship between the oligomycin-sensitive ATPase and the coupling mechanism of oxidative phosphorylation.

SUMMARY

1. An oligomycin-sensitive ATPase was isolated and partially purified from beef heart mitochondria. The specific activity of ATPase sensitive to oligomycin of the fraction was five to eight times that of aged mitochondrial or of DNP-induced mitochondrial ATPase assayed under the same condition.

2. Electron micrographs of the partially purified oligomycin-sensitive ATPase reveal a structure in which headpieces are regularly attached by way of stalks to a thread-like structure derived from a superficial portion of base pieces.

3. A high concentration of the structured material coincided with a high activity of oligomycin-sensitive ATPase. When the headpieces were detached from the structure, the ATPase became insensitive to oligomycin.

4. The fraction of oligomycin-sensitive ATPase was essentially free of mem-

brane structure and was contaminated with a small amount of cytochromes b and c_1 but no cyt. a. Cytochrome concentrations of the preparations were indifferent to the activity of oligomycin sensitive ATPase. It follows that ATPase does not require cytochromes or membrane structure for its oligomycin sensitivity.

5. From these results it seems that the factor rendering ATPase sensitive to oligomycin should be contained in the stalks and/or the thread-like portion of basepieces of the structure. The structure is the simplest unit of oligomycin-sensitive ATPase as yet obtained.

6. The structure was called "oligomycin-sensitive ATPase particles" (abbreviated as OSA particles). A unit of OSA particles consists of a headpiece attached by a stalk to a portion of base piece.

ACKNOWLEDGEMENT

This investigation was supported by research grants from the Ministry of Education, Japan and PHS research grant GM 10538, NIH, U. S. A. Our sincere thanks are due Mr. T. ITAYA for his technical assistance.

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