STUDIES ON THE MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE SYSTEM
V. LOCALIZATION OF THE OLIGOMYCIN-SENSITIVITY CONFERRING PROTEIN

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The cristael membrane of the mitochondrion has been shown to be composed of tripartite repeating units consisting of headpiece, basepiece and stalk (1). The headpiece has been identified with the oligomycin-insensitive ATPase complex (F_1) (2); the basepiece with any one of the complexes of the electron transfer chain (3); and the headpiece, in attachment to the stalk sector, with an oligomycin-sensitive form of the ATPase complex (4). The oligomycin-sensitivity conferring protein (OSCP) (5) has been shown not to be a component of the headpiece (5), and it is not a component of the electron transfer complexes. From such evidence it would follow that OSCP is localized in the stalk sector of the mitochondrial tripartite repeating unit.

The stalk which is visualized by electron microscopy is a cylindrical stem about 50 Å in length by 30 Å in diameter which links basepiece to headpiece (1). Since the stalk interdigitates with the basepiece at one end, and the headpiece at the other end, the stalk sector may contain, at each end, fitting parts which cannot readily be visualized by electron microscopy. Besides the headpiece, at least two components--OSCP and an insoluble factor--are required for oligomycin sensitivity (5,6), and both of these components must be localized in the stalk sector. It is not yet clear which component, OSCP or the insoluble factor, actually interacts with oligomycin. Our concern in this communication, however, is neither with the total number of protein species in the stalk sector nor with the exact geometry of the stalk sector, but rather with the identification of OSCP as the protein that makes up the cylindrical stalk.

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We have recently described the isolation of OSCP in homogeneous form and have estimated the molecular weight to be about 18,000 on the basis of determinations by the gel filtration method (5). Electron microscopic examination of OSCP (see Fig. 1) shows the protein to be a discrete unit with some tendency to form tetrads. The most reliable estimates of size and dimensions are made on the basis of measurements applied to the tetrads rather than to the monomeric species, and these measurements indicate that each unit is about 50± 5 Å in length and 30± 5 Å in diameter. The minimum molecular weight on the basis of these measurements is 17,000; the maximum is 40,000; and the mean is 27,000. The volume of the cylindrical stalk, computed on the basis of the dimensions determined electron micrographically (50 x 30 Å), matches very closely the volume of a protein 27,000 in molecular weight. Thus OSCP meets the dimensional requirements for identification with the protein making up the cylindrical stalk.

Another critical line of evidence relevant to this identification is provided by reconstitution of membrane bound ATPase activity by interaction of three components: (1) the residue of the ATPase complex after extraction sequentially with NaBr to remove headpieces, and with NH_4OH to remove OSCP; (2) headpiece (F_1); and (3) OSCP. In contrast to the ATPase activity of the unattached headpiece, the ATPase activity of the headpiece, after interaction with OSCP and the extracted residue, is sensitive to oligomycin (5) and to tributyl tin chloride, and is more specific for ATP as substrate (Table 1). The reconstituted ATPase activity is also more stable to cold than is the ATPase activity of the unattached headpiece (Fig. 2). When the extracted residue is mixed with the headpiece in absence of OSCP, no reconstitution of oligomycin-sensitive ATPase activity is found and no increase in specificity for ATP or loss of cold lability is observed. There is indeed interaction between the residue fraction and the headpiece, but the interaction in absence of OSCP does not lead to any change in the properties of the ATPase activity.

Examination by electron microscopy of the particles reconstituted from the

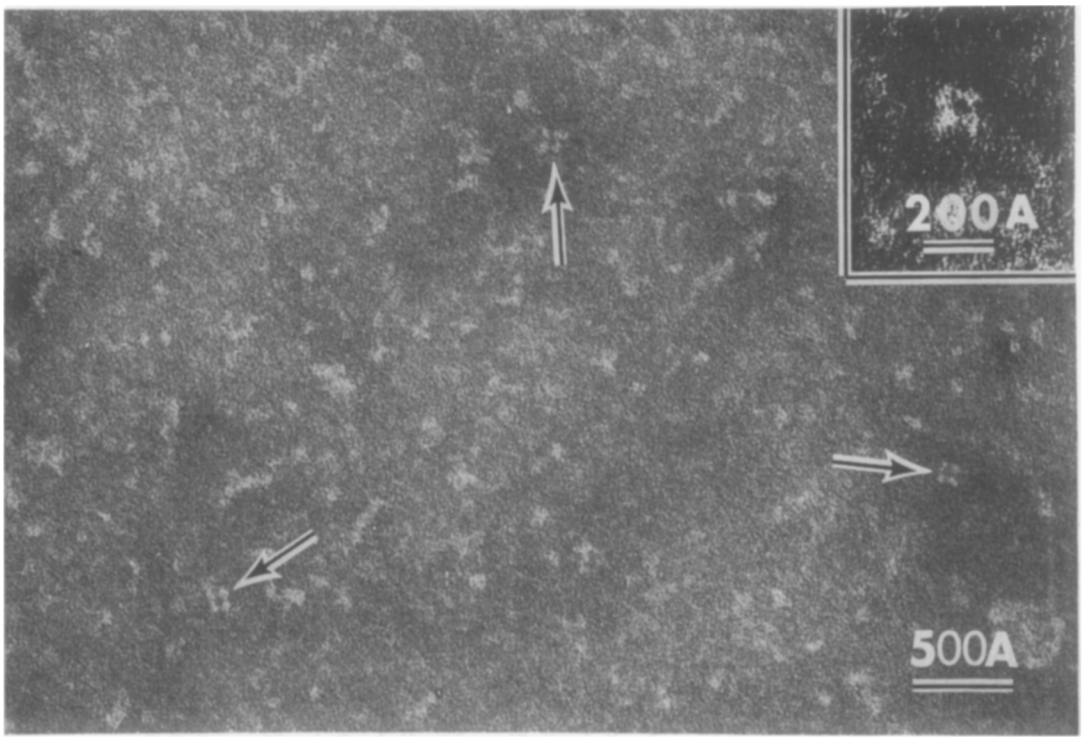

Fig. 1. Electron micrograph of a negatively stained sample of the purified oligomycin-sensitivity conferring protein.

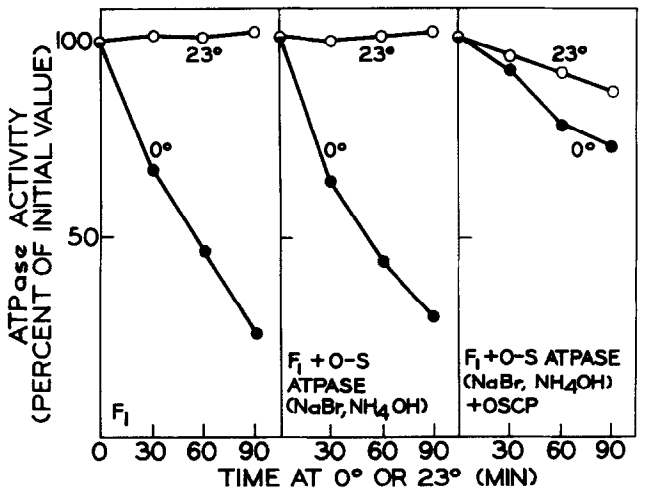

Fig. 2. Cold lability a) of F_1; b) of a combination of F_1 and the membraneous residue obtained after sequential extraction of the oligomycin-sensitive ATPase complex with NaBr and NH_4OH; c) of a combination of F_1, the membraneous residue, and OSCP. The experimental procedure was identical to that described elsewhere (6). One mg of particle was mixed with 200 µg of F_1 in presence or absence of 20 µg of OSCP. The initial ATPase specific activities were; a) 33.5; b) 2.70; c) 2.15.

Table I

Essentiality of OSCP for Nucleotide Specificity

Additions to F_1[1]	mμmoles P_i released per min per mg protein from:		$\frac{\text{Rate of GTP hydrolysis}}{\text{Rate of ATP hydrolysis}} \times 100$
	ATP	GTP	
No addition	28.8	34.0	118
O-S ATPase (NaBr)[2]	3.60	1.92	54
O-S ATPase (NaBr, NH_4OH)[3]	1.86	1.93	103
O-S ATPase (NaBr, NH_4OH), OSCP	2.00	1.40	70

1) 500 μg of F_1 was incubated for 10 min at 23° with 2.5 mg of the extracted ATPase complex and 75 μg of OSCP. The suspending medium was 1.0 ml of a solution 0.25 M in sucrose, and 0.01 M in Tris-SO_4, pH 7.5. The particles were collected by centrifugation, suspended in 0.25 ml of sucrose-Tris, and 0.1 ml was taken for assay of nucleotidase activity. The assay mixture was 1.0 ml of a solution 5 mM in $MgCl_2$, 50 mM in Tris-SO_4, pH 7.5, and 10 mM in triphosphonucleotide.

2) O-S ATPase (NaBr) is the residue obtained after extraction of the oligomycin-sensitive ATPase complex with a solution 3.5 M in NaBr. The residue is devoid of headpieces and of ATPase activity but is not depleted of OSCP.

3) O-S ATPase (NaBr, NH_4OH) is the residue obtained after sequential extraction of the oligomycin-sensitive ATPase complex first with 3.5 M NaBr and then with 0.4 M NH_4OH. The preparation so obtained is devoid of headpieces and depleted of OSCP.

three components as described above reveals tripartite repeating units in profusion (Fig. 3C). When the extracted residue is allowed to react with the headpiece in the absence of OSCP the resulting membraneous particles show only occasional tripartite structures, and most of the added headpieces can be distinguished as units unattached to the membrane (Fig. 3B). These morphological observations clearly point to OSCP as the structural unit which specifically links headpiece to basepiece in the tripartite unit. It is important to dis-

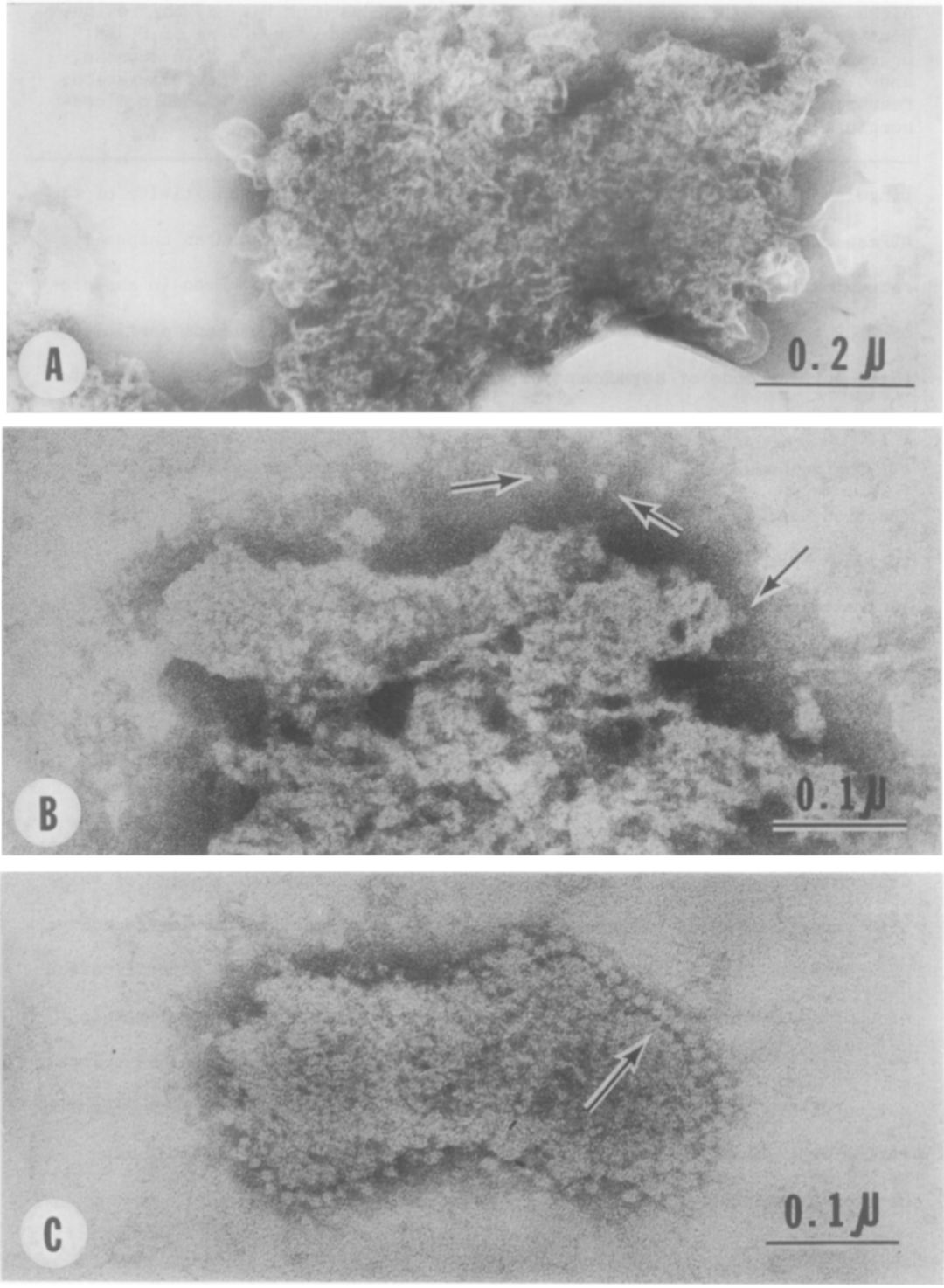
A
0.2 μ
B
0.1 μ
C
0.1 μ

Fig. 3. Electron micrographs of negatively stained preparations. a) the residue obtained after sequential extraction of the oligomycin-sensitive ATPase complex with NaBr and NH_4OH. b) the residue plus F_1. c) the residue plus F_1 plus OSCP. One mg of particle was incubated with 200 µg of F_1 in presence or absence of 20 µg of OSCP in 1 ml of a medium 0.25 M in sucrose, and 0.01 M in Tris-SO_4, pH 7.5. Particles were collected by centrifugation, resuspended in sucrose-Tris, and negatively stained with PTA prior to microscopic examination.

tinguish between a recombination which leads to oligomycin-sensitivity of the ATPase function and the emergence of tripartite structure, and an unspecific recombination which leads to neither of these consequences. Even in absence of OSCP the headpieces can combine with the extracted membraneous particles (5,6) but the mode of attachment of the two is abnormal and does not lead to emergent properties.

The membraneous particle which was the starting point for the residue fraction used in the above-mentioned reconstitutions was exposed successively to 3.5 M NaBr and 0.4 M NH_4OH. The headpieces were found to be physically separated from the membraneous particles in consequence of this treatment. The fitting end of the stalk which interdigitates with the basepiece was not dislodged by either treatment and only part of the OSCP was brought into solution during extraction with NH_4OH. Despite its persistence in the ammonia-treated particles, as determined by gel electrophoresis, this residual OSCP was found to be functionally incompetent. For all practical purposes OSCP dislocated by alkaline extraction was unavailable for reconstitution of tripartite structure. Only exogenously added OSCP could function in that capacity.

There are thus several lines of evidence which support the identification of OSCP as the protein which makes up the cylindrical stalk. The first is the evidence of molecular size and shape; the second, that of exclusive localization within the stalk sector (4); and the third, the evidence from reconstitution experiments that OSCP is required to reestablish the physical link between basepieces and headpieces.

The localization of OSCP in the cylindrical stalk of the tripartite unit would suggest a role for this protein as the molecular instrument for the

intercommunication of two energizing systems; the ATPase system in the headpiece and the electron transfer system in the basepiece. The capability for conferring oligomycin-sensitivity on the ATPase function impels us to consider OSCP as the protein which meshes the ATPase function with the work performances of the inner membrane.

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