THE SUBUNIT COMPOSITION OF THE MITOCHONDRIAL OLIGOMYCIN-INSENSITIVE ATPase*

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1. Introduction

In continuation of our previous work [1], we have examined several different preparations of soluble mitochondrial oligomycin-insensitive ATPase, from both rat liver and beef heart, by the technique of polyacrylamide gel electrophoresis in SDS. The results suggest that the mitochondrial "headpiece" contains at least six different types of subunit. A tentative subunit composition is suggested.

2. Materials and methods

Preparation of ATPase enzyme. ATPase enzyme from beef heart mitochondria was purified by each of the following procedures: (A) the procedure of Senior and Brooks [1] taken through step 5; (B) the procedure of Horstman and Racker [2]; (C) the procedure of Datta and Penefsky [3]; (D) from an acetone-powder of beef heart mitochondria. An acetone powder was prepared [4] and extracted by homogenization at 100 mg protein/ml at room temperature in a solution 40 mM in Tris-SO_4, 1 mM in EDTA and 2 mM in ATP, pH 7.4. The homogenate was centrifuged at 105,000 *g* for 20 min, the supernatant was retained, and the pellet material was re-extracted three times. Soluble ATPase enzyme of specific activity $\geqslant 100$ was purified from the supernatants by ammonium sulfate fractionation, ion-exchange chromatography and Sephadex G-200 gel filtration as described previously [1]. ATPase enzyme from rat liver mitochondria (specific activity = 100) was a gift of Dr. David Lambeth, prepared by a technique involving sonication, protamine sulfate fractionation and ion-exchange chromatography on DEAE-Sephadex [5].

Polyacrylamide gel electrophoresis. SDS-gel electrophoresis in 5%-gels was carried out as previously described [1]. SDS-gel electrophoresis in 10%-gels, prepared as described by Dunker and Rueckert [6], was carried out for 3 hr at 8 V/cm of gel. Gels were soaked 18 hr in 10% CCl_3COOH before staining with Coomassie Blue for 8 hr [1].

Analytical methods. Protein estimations and ATPase assays were carried out as described previously [1].

3. Results

Dissociation of the ATPase enzyme complex in 5%-gels in SDS. As we described previously [1] the enzyme complex prepared by the technique of Senior and Brooks dissociates into four types of subunit in 5%-gels in SDS. We have succeeded in purifying each subunit and chemical analyses have shown that the four types of subunit are chemically distict [7]. A typical densitometer tracing of a 5%-gel is shown in fig. 1. It is to be noted that the heaviest component accounts for a large proportion of the total stain. Indeed, the two small components are only seen clearly when 25 μg or more protein is applied to the gel.

All of the other enzyme preparations examined

Abbreviation:
SDS: sodium dodecyl sulfate.

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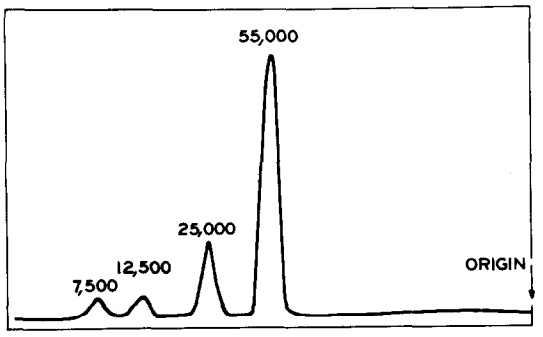

Fig. 1. Electrophoresis of soluble mitochondrial ATPase (30 μg) in 5%-gel in SDS. This is a densitometric trace of the gel, migration was from the origin at the right toward the anode at the left. Calculated molecular weights of components are shown.

here (see sect. 2) exactly resembled the preparation of Senior and Brooks, both in number and relative proportion of subunits, with the exception of the preparation of Horstman and Racker (prep. B). This last preparation contained the four subunits contained in the other preparations, and in the same proportions, but, additionally, contained a fifth component of molecular weight 10,500. This component has been identified as the specific mitochondrial ATPase inhibitor protein [7].

Dissociation of the ATPase-enzyme in 10%-gels in SDS. When the various preparations of enzyme were examined in 10%-gels in SDS a further feature was revealed. The heaviest component, common to all preparations, was resolved into two discrete components, quite similar in molecular weight (see fig. 2).

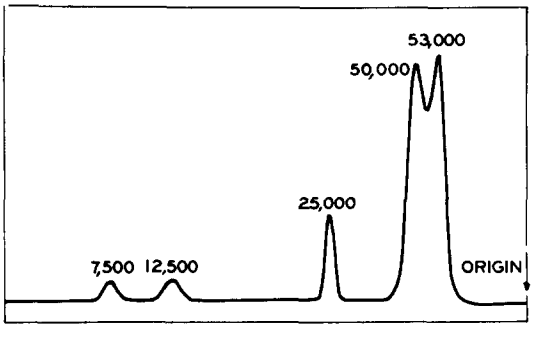

Fig. 2. Electrophoresis of soluble mitochondrial ATPase (30 μg) in 10%-gel in SDS. See fig. 1 for details.

The molecular weights calculated from 10%-gels are probably more reliable than those in 5%-gels. Again, all preparations examined resembled the Senior and Brooks enzyme (as in fig. 2) with the exception of the preparation of Horstman and Racker which contained, additionally, a component of M.W. = 10,500, identified as the inhibitor protein (see above).

4. Discussion

The technique of SDS-gel electrophoresis reveals the presence of several subunits in preparations of mitochondrial ATPase. In 10%-gels, which gave the maximum resolution, we noted the presence of subunits of molecular weight 53,000, 50,000, 25,000, 12,500 and 7500 in all preparations examined. The preparation of Horstman and Racker contained an additional sixth component of molecular weight 10,500, which is the specific mitochondrial ATPase inhibitor protein.

These results indicate therefore that the mitochondrial "headpiece" contains six different types of subunit. The question arises, are the subunit components of the ATPase enzyme normally present in the headpiece in the natural state, or is the complex subunit strcuture indicated merely an artifact of the techniques of purification? We cannot answer this question definitively. But the facts that the purification techniques used would effectively separate these components were they not tightly bound together, and that the same subunits in the same proportions are always seen in preparations from different tissue and prepared by different techniques, suggest to us that the enzyme contains these components in the natural state. The inhibitor protein appears to be more easily removed than the other components, but there seems little doubt in view of its specific ability to inhibit ATPase activity that it originates from the headpiece.

The total number of subunits remains unknown at this time. We have failed to detect *N*-terminal amino acids and have not completed *C*-terminal analyses. However, it is interesting that a multimeric structure consisting of one subunit each of molecular weight 7500, 10,500, 12,500 and 25,000, with six subunits each of about 51,500 molecular weight, would have a total molecular weight of 364,500. This proportionality

betweem subunits would be approximately consistent with the proportions seen in tracings of gels. As noted in the preceding communication [8], the molecular weight of soluble mitochondrial ATPase from both rat liver [5, 8] and beef heart [8] appears to be about 360,000 and not 284,000 as previously stated [9, 10].

Finally, it is worthy of note that when the mitochondrial ATPase is depolymerized in 6 M guanidine hydrochloride and chromatographed on Sephadex G-200 in the same buffer, the elution pattern indicates the presence of several subunits of different molecular weight, and is consistent with the results obtained by gel electrophoresis in SDS (J.C. Brooks, unpublished observations). Thus the recent subunit structure proposed by Forrest and Edelstein [9], which was based on ultracentrifuge studies of ATPase dissolved in 6 M guanidine hydrochloride, appears less complete than the structure we propose here because of the inability of the ultracentrifugation technique to clearly resolve components of a multi-component system.

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