ON THE SUBUNIT STRUCTURE OF SOLUBLE MITOCHONDRIAL ATPase

I. A. KOZLOV and H. N. MIKELSAAR

Laboratory of Bioorganic Chemistry, Moscow State University, Moscow 11734, USSR; Institute of Experimental Biology of the Estonian Academy of Sciences, Tallinn, USSR

Received 9 April 1974

1. Introduction

The study of the structure and mechanism of action of soluble mitochondrial ATPase (coupling factor F_1) answers a number of questions about the functioning of this enzyme in the oxidative phosphorylation system.

ATPase is made up of several subunits [1–4] and the fact that it has a complex subunit structure is in agreement with the suggestions of several authors [5–9] on the energy-dependent changes in the conformation of the enzyme in the membrane which occur when ATP is formed or hydrolysed. This raises two important questions: 1) To what extent does the conformation of ATPase depend on its specific environment in the mitochondrial membrane? 2) What are the roles of the individual subunits of ATPase in the interaction of the enzyme with the membrane, in the hydrolysis of ATP and in the specific sorption of ADP? These questions point to the significance of a study of the subunit structure of soluble mitochondrial ATPase.

2. Methods

ATPase was obtained according to Raker [10] from beef heart mitochondria. Electrophoresis in 10% polyacrylamide gel, in the presence of sodium dodecyl sulphate (SDS) was done according to Weber and Osborn [11]. The rate of the ATPase reaction was followed by the change in the pH of the reaction mixture [12]. The reaction was carried out in a mixture of the following composition: 0.15–5.0 mM ATP; 0.3–10 mM $MgSO_4$; 0–2.5 mM ADP; 5–15 μg enzyme; 3 mM, Tris–HCl buffer, pH 8.3 in a total volume of 8 ml. In the experiments on the dependence of the reaction rate on the concentration of the substrate, the ratio [Mg-ATP]/[Mg^{2+}] free = 1, remained constant. During the study of the inhibition of the ATPase reaction by Mg-ADP there was a constant excess of 1 mM free Mg^{2+}. The results of the kinetic measurements are presented as Lineweaver–Burk and Dixon plots. All the kinetic measurements were made at 25°C.

3. Results and discussion

The electrophoresis of a preparation of ATPase obtained by the standard method of Racker [10] in a 10% polyacrylamide gel in the presence of 0.2% of SDS led to the appearance of 5 protein bands. The results obtained correspond completely to the data in the literature [1,2,4,13]. At present the opinion is expressed in the literature that these five protein bands correspond to 5 different subunits of ATPase. Knowles and Peneffsky [1] have given the molecular weights of the individual subunits, determined by several methods. These are: 54 000 (α) 50 000 (β) 33 000 (γ); 16 000 (δ) and 11 000 (ϵ). The last of these subunits is a dimer of a protein inhibitor of ATPase [1]. The molecular weights reported by Knowles and Penefsky are basically in agreement with the data of other authors [2,4,13].

In order to study the functional role of the ATPase subunits of molecular weights of 33 000 and 16 000, we isolated, in addition to the standard preparation of F_1, an enzyme which was shown by SDS gel electrophoresis not to contain the γ and δ components (fig. 1). This preparation of ATPase, lacking the

North-Holland Publishing Company – Amsterdam

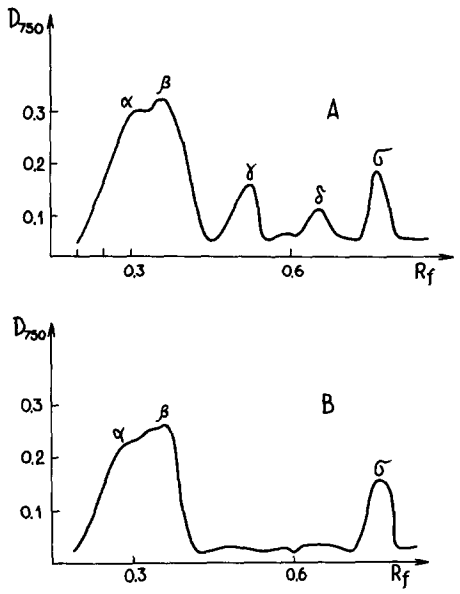

Fig. 1. Electrophoresis of ATPase in polyacrylamide gel in the presence of SDS: (A) standard preparation of F_1; (B) preparation of F_1 without the γ and δ components. 70 μg of protein were applied on the gel. The samples were stained with Amido Black. The electrophoretic mobility of the individual protein components was calculated with reference to the mobility of bromophenol blue.

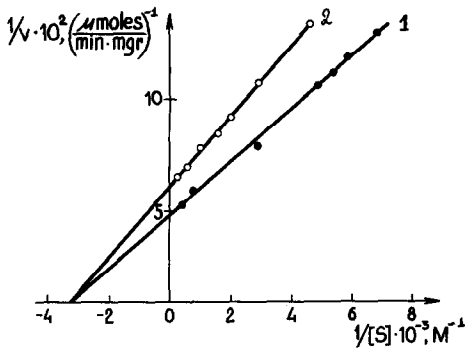

Fig. 2. The dependence of the reciprocal rate of the ATPase reaction on the reciprocal concentration of the substrate: (1) standard preparation of F_1; (2) F_1 without the γ and δ minor components. The composition of the reaction medium: 3 mM Tris–HCl buffer; pH 8.3; 5–15 enzyme μg; Mg^{2+} and AT as above. ([Mg-ATP]/[Mg^{2+}] free = 1) in a total volume of 8 ml.

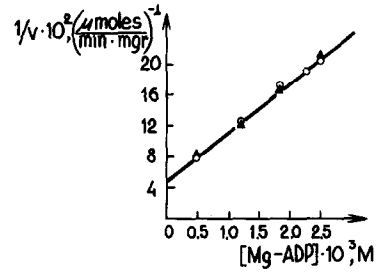

Fig. 3. The dependence of the reciprocal rate of the ATPase reaction on the concentrations of Mg-ADP: (○) – standard preparation of F_1; (▲) – F_1 without the γ and δ minor components. The composition of the reaction medium: 3 mM Tris–HCl buffer, 3 mM, pH 8.3, 5–15 μg enzyme; 2 mM Mg-ATP. Mg-ADP as above, Mg^{2+} free 1 mM in a total volume of 8 ml.

γ and δ subunits, may be obtained if a suspension of mitochondria undergoes sonication at pH 5.2. In the subsequent steps of the isolation of the enzyme, Racker's procedure was exactly followed [10].

A comparison of the catalytic properties of the ATPase's containing and not containing the γ and δ subunits, showed that both preparations have similar $K_{m\ (app)}$ and V_{max} values and that their affinity for the inhibitor of the ATPase reaction, ADP, is identical (figs. 2, 3). Thus, the γ and δ subunits do not, in all probability, take part in the catalytic act of the ATPase reaction. These results and also the available data in the literature allow us to make certain suggestions regarding the functional role of the γ and δ subunits of the coupling factor.

Having measured the intensity of the protein bands in polyacrylamide gel and having taken into consideration the contribution of the molecular weight of each subunit, Senior and Brooks [2] and Catterall and Pedersen [3] came to the conclusion that each molecule of ATPase has 6 large subunits (3+3) and several minor ones, one of each type. It should be noted that the sum of the molecular weights of the minor subunits (with or without the protein inhibitor monomer) is very similar to the molecular weight of the major subunits. This led us to suggest that a molecule of mitochondrial ATPase consists of seven protein globules. The authors of [14] discerned a similar structure (a planar hexagon, inside which there is the seventh globule) in an electron micrograph of ATPase from *Micrococcus* membrane.

The appearance of the γ and δ subunits is perhaps

the consequence of the enzymic cleavage of the seven protein globules into two polypeptides. The single rupture in the protein chain does not immediately show up due to internal forces which maintain the secondary, yeriary and quaternary structure of F_1. When SDS is added, disassociation of the two polypeptide chains making up the seventh globule, is observed.

Knowles and Penefsky [1] and also Senior and Brooks [15] have shown a considerable similarity between the amino acid compositions of the α and β subunits of ATPase. On the other hand, there is as substantial difference between the amino acid compositions of the α and β subunits and those of the γ and δ subunits. The calculation made by us of the amino acid composition of the hypothetical seventh globule (the total amino acid content of the γ and δ subunits), on the basis of literature data, revealed the striking similarity of that globule to the α and β subunits of ATPase.

The functional role of the seventh globule is, in our opinion, connected with the existence of linkages between ATPase and the membrane. A similar idea has been put forward in the works of Salton et al. [16]. The authors obtained *Micrococcus* factor F_1 of two types, one of which did not contain the minor components in SDS polyacrylamide gel and was not able to interact with the membrane. The second possessed these components and was able to bind with the bacterial membrane.

Nor can it be excluded that the seventh globule takes part in the mechanism of energy coupling (for instance, in the formation of the initial high energy intermediate, the translocation of the proteins across the membrane and so forth). Thus, in accordance with our recent hypothesis [6], the seventh globule which is to be found, in one of the conformations of ATPase, within the hexagon may occur in another conformation, i.e. as a stalk of the so-called knobs of membrane ATPase (two of the possible conformations of the subunits of factor F_1 given in fig. 4).

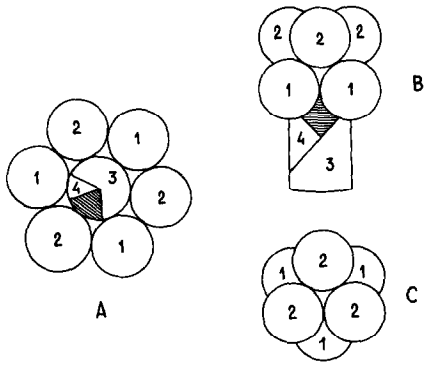

Fig. 4. The two conformations of mitochondrial ATPase (A) the planar hexagon with the seventh central globule (B and C) a knob- with the seventh globule as the so called 'stalk' (view from the side and from above).

Acknowledgements

The authors would like to thank Dr V. P. Skulachev for his valuable advice in discussing the results of this work, and also Miss V. A. Kononenko for her assistance

References

[1] Knowles, A. F. and Penefsky, H. S. (1972) J. Biol. Chem. 247, 6617–6624.
[2] Senior, A. E. and Brooke, J. C. (1971) FEBS Letters 17, 327.
[3] Catterall, W. A. and Pedersen, P. L. (1971) J. Biol. Chem. 246, 4987.
[4] Van de Stadt, R. J., Kraaipoel, R. J. and Van Dam, K. (1972) Biochim. Biophys. Acta 267, 25.
[5] Ryrie, I. J. and Jagendorf, A. T. (1972) J. Biol. Chem. 247, 4453.
[6] Mikelsaar, N. H. (1972) Dokl. Akad. Nauk SSSR 203, 704.
[7] Racker, E. (1972) Federation Proc. 31, 10.
[8] Hatase, O. and Oda, T. (1972) J. Biochem. 71, 759.
[9] Hatase, O., Wakabayashi, T., Hayashi, H. and Green, D. E. (1972) J. Bioenergetics 3, 509.
[10] Racker, E. and Horstman, L. L. (1967) J. Biol. Chem. 242, 2547.
[11] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406.
[12] Nishimura, M., Ito, T., Chance, B. (1962) Biochim. Biophys. Acta 52, 177.
[13] Tzagoloff, A. and Meagher, P. (1971) J. Biol. Chem. 246, 7328.
[14] Munoz, E., Freer, J. H., Ellar, D. J. and Salton, M. R. J. (1968) Biochim. Biophys. Acta 150, 531.
[15] Brooks, J. C., Sendor, A. E. (1972) Biochemistry 11, 4675.
[16] Salton, M. R. J. and Schor, M. T. (1972) Biochem. Biophys. Res. Commun. 49, 350.