Biochimica et Biophysica Acta, 1015 (1990) 49–52
Elsevier

BBABIO 43118

On the subunit composition of plant mitochondrial ATP synthase

Birgitta Norling, Christos Tourikas, Beston Hamasur and Elzbieta Glaser

Department of Biochemistry, Arrhenius Laboratories for Natural Sciences, University of Stockholm, Stockholm (Sweden)

(Received 26 May 1989)

Key words: Cross-reconstitution; ATPase, F_1-; ATP-synthase; Oligomycin sensitivity; Mitochondrion; (Plant)

Cross-reconstitution experiments using potato mitochondrial F_1 and beef heart submitochondrial particles depleted of F_1 or F_1 and OSCP were performed. F_1 conferred oligomycin sensitivity on F_1-depleted particles (75–85%), whereas only very low oligomycin sensitivity was observed with F_1 and OSCP-depleted particles (approx. 20%). These findings show that potato mitochondrial F_1 recognizes mammalian OSCP for the formation of an oligomycin-sensitive enzyme, which indicates the existence of an OSCP-like protein in the plant mitochondrial ATP-synthase.

Introduction

Proton-translocating ATP synthases of mitochondria, chloroplasts and bacteria have basically similar structure and function (for recent reviews see Refs. 1–4). These enzymes are composed of two structurally and functionally distinct units, a hydrophilic peripheral part, F_1 containing the catalytic site of the enzyme, and a hydrophobic membrane-integrated part, F_0, constituting the H^+-translocating moiety of the enzyme. Both ATP synthesis and hydrolysis are coupled to H^+-translocation through F_0. Oligomycin, an inhibitor of ATP synthesis/hydrolysis catalyzed by the mitochondrial enzyme, exerts its effect by binding to F_0 and blocking H^+-translocation through F_0. Oligomycin resistance-conferring mutations have been found in subunits a and c of F_0 in yeast [5–7] and subunit a in Chinese hamster ovary cells [8]. Missense mutations affecting proton-conduction rates or function of F_0F_1 are found in the same regions of subunits a and c (for review see Ref. 1).

For an understanding of the mechanism of ATP synthesis, the elucidation of the mode of coupling between the catalytic part F_1 and the proton channel F_0 is of great importance. On the basis of reconstitution experiments it was concluded [9–12] that, in the ATP synthase of yeast and mammalian mitochondria, the oligomycin sensitivity conferring protein (OSCP) is one of the peptides that links F_1 to the membrane and provides a correct subunit organization of the enzyme complex. No OSCP is present in bacterial or chloroplast F_0F_1-ATPases, but it was recognized by Walker et al. [13] that the δ subunit of *Escherichia coli* is homologous to beef heart OSCP. Later it was found that the δ-subunit of F_0F_1 from spinach chloroplast [14] and from the phototrophic bacteria *Rhodospirillum rubrum* [15] show homologies with OSCP. However, only *R. rubrum* is sensitive to oligomycin [16]. It has also been shown that *R. rubrum* F_1-ATPase with the β-subunits substituted with β from *E. coli* is not oligomycin sensitive when reconstituted to F_1-depleted membranes [17]. Thus it is obvious that also the structure of the β-subunit is crucial for oligomycin sensitivity.

Whereas the bacterial, yeast and mammalian F_0F_1-complexes have thus been extensively characterized concerning oligomycin sensitivity, limited information is available for the plant mitochondrial enzyme in this respect. The catalytic part F_1, on the other hand, has been purified from a few plant sources [18–22]. No reports have been presented concerning the composition and properties of the H^+-translocating part, F_0. So far no one has been able to prepare plant submitochondrial particles completely devoid of F_1 which are reconstitutively active when recombined with the homologous plant F_1. It has been reported that addition of F_1 to partially F_1-deficient pea cotyledon submitochondrial particles increases the rate of ATP synthesis [23]. Most of the increase was, however, due to a structural rather than a catalytic effect of added F_1, which provided a block in the proton pore of F_0, thus enabling the residual F_1 to utilize the increased proton gradient.

We have earlier reported [24] cross-reconstitution experiments between F_1 isolated from potato tuber mitochondria and F_1-depleted submitochondrial par-

Abbreviation: OSCP, oligomycin sensitivity conferring protein.

Correspondence: B. Norling, Dept. of Biochemistry, Arrhenius Laboratories for Natural Sciences, University of Stockholm, S-106 91, Stockholm, Sweden.

ticles from beef heart as well as from yeast. It was found that potato F_1 binds to both types of heterologous membrane in amounts similar to those originally present, giving rise to an oligomycin-sensitive ATPase, indicating a close relationship between the mitochondrial enzyme of plant, mammals and yeast. The present work is an extension of these studies in order to investigate further the possible involvement of an OSCP-like protein in the organization of the plant enzyme.

Material and Methods

F_1 from potato tuber mitochondria was isolated as described in Ref. 22. Beef heart submitochondrial particles were depleted of F_1 by urea treatment [25] and subsequently depleted of OSCP by ammonia treatment [26].

Binding of potato F_1 to F_1-depleted heart submitochondrial particles and to F_1-OSCP-depleted particles was performed as follows. Various amounts of isolated potato F_1 were incubated with beef heart membranes (0.8 mg/ml) for 30 min at room temperature in a medium containing 0.25 mM sucrose, 10 mM Tris-sulphate (pH 8.0), 0.25 mM EDTA and 9 mM $MgCl_2$. Bound and unbound F_1 were separated by centrifugation in an Eppendorf table centrifuge.

ATPase activity was measured by coupling the reaction to the pyruvate kinase and lactate dehydrogenase reactions and following the oxidation of NADH at 340 nm [27].

Protein was determined by the method of Peterson et al. [28].

Results and Discussion

Urea treatment of beef heart submitochondrial particles results in a virtually complete depletion of F_1, as judged by the residual ATPase activity, being less than 0.5% of the original. Addition of purified been-heart F_1 to these particles restores energy-dependent reactions and oligomycin-sensitive ATPase activity [29]. These particles can also bind isolated potato F_1 and confer oligomycin sensitivity on the bound F_1, indicating that a functional ATPase complex is formed between the plant and the animal parts of the enzyme. After reconstitution, the total activity of the soluble potato F_1 added is recovered in the particles and in the supernatant, which contains F_1 that is not bound. By increasing the amount of potato F_1 during reconstitution, more F_1 can be bound, and as seen in Fig. 1 (open circles) maximally about 10 μg potato F_1 is bound per 100 μg F_1-depleted beef heart membranes. This amount is similar to that found in reconstitution experiments with beef heart F_1 and beef heart membranes [30]. It can also be seen in Fig. 1, that potato F_1 has a very high affinity for the heterologous membranes, since below 5 μg F_1 added per

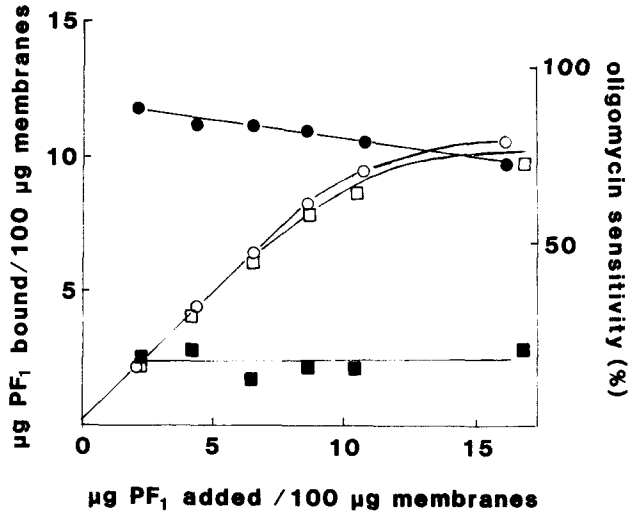

Fig. 1. Binding of potato F_1 to F_1-depleted beef heart submitochondrial particles and to F_1-OSCP-depleted particles and conferal of oligomycin sensitivity. Potato F_1 bound to F_1-depleted membranes (○) and conferral of oligomycin sensitivity (●). Potato F_1 bound to F_1-OSCP-depleted membranes (□) and conferral of oligomycin sensitivity (■). ATPase activity was measured as described in Material and Methods. Oligomycin, 3 nmol/mg protein, was added directly to the cuvette during the ATPase assay.

100 μg membranes, virtually all F_1 is bound. The slightly lower oligomycin sensitivity shown at higher ratios of F_1 to membranes may indicate that under these conditions some nonspecific binding of F_1 is obtained.

As seen in Fig. 2, cations are required for binding of potato F_1 to F_1-depleted beef heart particles (open circles). Optimal binding is obtained at about 7 mM

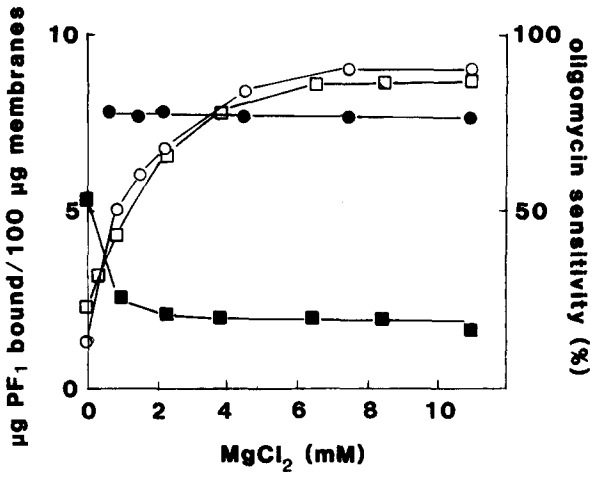

Fig. 2. Effect of divalent cations on the binding of potato F_1 to F_1-depleted beef heart submitochondrial particles and to F_1-OSCP-depleted particles and conferral of oligomycin sensitivity. Reconstitutions were performed with 13 μg F_1/100 μg membranes and with the indicated concentrations of $MgCl_2$ in the reconstitution medium. Further conditions as described in Material and Methods. F_1 bound to F_1-depleted membranes (○) and conferral of oligomycin sensitivity (■). F_1 bound to F_1-OSCP-depleted membranes (□) and conferral of oligomycin sensitivity (■).

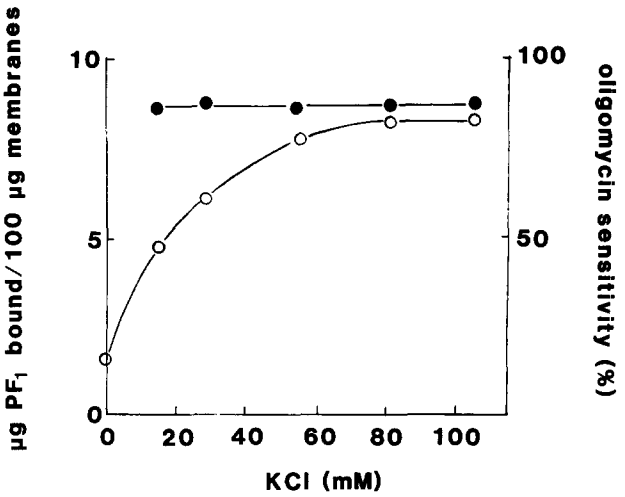

Fig. 3. Effect of monovalent cations on the binding of potato F_1 to F_1-depleted beef heart submitochondrial particles and conferral of oligomycin sensitivity. Reconstitutions were performed with 9 μg F_1/100 μg membranes and the indicated concentrations of KCl. Further conditions as described in Material and Methods. F_1 bound to the membranes (○) and conferral of oligomycin sensitivity (●).

$MgCl_2$. Although less F_1 is bound at suboptimal concentrations of $MgCl_2$, the same sensitivity to oligomycin is obtained, indicating that no cations, or much lower concentrations, are needed for conferral of oligomycin sensitivity. Other divalent cations tested are Ca^{2+}, Mn^{2+} and Ba^{2+}, which all give similar titers for optimal binding and conferral of oligomycin sensitivity. Fig. 3 demonstrates that also the monovalent cation, K^+, induces binding of potato F_1 to F_1-depleted beef heart submitochondrial particles and concomitant conferral of oligomycin sensitivity. About 80 mM of the monovalent cation is, however, required for optimal binding.

These results concerning cation requirement in cross-reconstitution are in accordance with results reported on reconstitution experiments involving both F_1 and F_1-depleted membranes from beef heart particles [31]. Ca^{2+} and Mg^{2+} at 5 mM as well as K^+, NH_4^+ and Na^+ at 50 mM were found to give optimal binding of F_1. Furthermore, it was demonstrated that at corresponding concentrations of Na^+ and Ca^{2+}, the negative ξ-potential of the particles was decreased to zero. The effect of the cations can be interpreted in terms of overcoming the repulsion of the negative membrane surface charges on F_1, which also is negatively charged.

It is known for the ATPase synthase of both yeast [12] and beef heart [11] that F_1 can bind to F_0 in the absence of OSCP, provided cations are present. In this case, however, conferral of oligomycin sensitivity is abolished as well as energy-dependent reactions. On the other hand, if F_1- and OSCP-depleted submitochondrial particles were supplemented with the purified coupling factors (F_1 and OSCP), energy-dependent ATP-P_i exchange and energy-linked transhydrogenase could be fully restored [9]. This indicates that the treatments with urea and ammonia do not remove or destroy other components of the enzyme complex. Urea- and ammonia-treated beef-heart submitochondrial were used in the present work in cross-reconstitution experiments with purified potato F_1. In Fig. 1 it is seen that similar amounts of potato F_1 are bound in the absence of OSCP (open squares) as in the presence of OSCP (open circles) indicating stoichiometric binding of F_1 to F_0. However, as expected, oligomycin sensitivity is abolished in the absence of OSCP (Fig. 1, filled squares). The low oligomycin sensitivity found (approx. 20%) is due to the presence of residual OSCP in the particles [32].

There is also a cation dependency in binding of potato F_1 to beef heart particles in the absence of OSCP (Fig. 2, open squares). In the same figure (filled squares) it can furthermore be seen that in the absence of cations binding of small amounts of potato F_1 to F_1-OSCP-depleted particles results in a significantly higher degree of conferral of oligomycin sensitivity (approx. 50%) than binding of greater amounts in the presence of $MgCl_2$. A plausible explanation for this is that, in the absence of cations, F_1 has a higher affinity for those F_0 containing OSCP (approx. 20% residual) than for those devoid of OSCP. The isoelectric point of OSCP is high, 9.5 [33], which gives it a positive charge at the pH employed, which may attract F_1. For the beef heart enzyme it is also known that OSCP binds to F_1 at a specific binding site on the α and/or β subunit [32].

Some preparations of plant mitochondrial F_1 [19–21] have been reported to contain an additional component, δ′, in addition to α, β, γ, δ and ε. This additional component has been suggested to be related to the mammalian OSCP [34]. No experimental evidence for this hypothesis was, however, presented. The potato F_1 used in the present communication does not contain δ′ [35]. In summary, the present work shows that this five-subunit potato F_1 can be used in cross-reconstitution experiments with two distinct different types of beef heart submitochondrial particle, i.e., F_1-depleted and F_1-OSCP-depleted. It is clearly revealed by these cross-reconstitution experiments that potato F_1 behaves very similarly to beef heart F_1 with respect to both types of membrane. These similarities include amount of F_1 bound, cation requirement and conferral of oligomycin sensitivity. Potato F_1 recognizes beef heart OSCP in the formation of a reconstitutively active enzyme, indicating that an OSCP-like protein is involved also in the organization of the plant ATP synthase. By the use of oligonucleotide probes of the mammalian OSCP, the clone for plant OSCP might be found and identified.

Acknowledgements

This work was supported by research grant from the Swedish Natural Science Research Council, Carl

Tryggers Foundation and O.E. and Edla Johanssons Foundation.

References

1 Senior, A.E. (1988) Physiol. Rev. 68, 177–231.
2 Amzel, L.M. and Pedersen, P.L. (1983) Annu. Rev. Biochem. 52, 801–824.
3 Vignais, P.V. and Satre, M. (1984) Mol. Cell. Biochem. 60, 33–70.
4 Hatefi, Y. (1985) Annu. Rev. Biochem. 54, 1015–1069.
5 Macino, G. and Tzagoloff, A. (1980) Cell 20, 507–517.
6 Nagley, P.R., Hall, R.M. and Ooi, B.G. (1986) FEBS Lett. 195, 159–163.
7 Jean-François, M.J.B., Hadikusomo, R.G., Watkins, L.C., Lukins, H.B., Linnane, A.W. and Marzuki, S. (1986) Biochim. Biophys. Acta 852, 133–143.
8 Breen, G.A.M., Miller, D.L., Holmans, P.L. and Welch, G. (1986) J. Biol. Chem. 261, 11680–11685.
9 MacLennan, D.U. and Tzagoloff, A. (1968) Biochemistry 7, 1603–1610.
10 MacLennan, D.U. and Asai, J. (1968) Biochem. Biophys. Res. Commun. 33, 441–447.
11 Kagawa, Y. and Racker, E. (1971) J. Biol. 246, 5477–5487.
12 Tzagoloff, A. (1970) J. Biol. Chem. 245, 1545–1551.
13 Walker, J.E., Fearnley, I.M., Gay, N.G., Gibson, B.W., Northrop, F.D., Powell, S.J., Runswick, M.J., Saraste, M. and Tybulewicz, V.L.J. (1985) J. Mol. Biol. 184, 677–701.
14 Hermans, J., Rother, Ch., Bichler, J., Steppuhn, J. and Herrmann, R.G. (1988) Plant Mol. Biol. 10, 323–330.
15 Falk, G., Hampe, A. and Walker, J.E. (1985) Biochem. J. 228, 391–407.
16 Baltscheffsky, M. and Baltscheffsky, H. (1960) Acta Chem. Scand. 14, 257–263.
17 Gromet-Elhanan, Z., Khananshvili, D., Weiss, S., Kanazawa, H. and Futai, M. (1985) J. Biol. Chem. 260, 12635–12640.
18 Hack, E. and Leaver, C.J. (1983) EMBO J. 2, 1783–1789.
19 Boutry, M., Briquet, M. and Goffeau, A. (1983) J. Biol. Chem. 258, 8529–8536.
20 Iwasaki, Y. and Asahi, T. (1983) Arch. Biochem. Biophys. 227, 164–173.
21 Horak, A. and Packer, M. (1985) Biochim. Biophys. Acta 810, 310–318.
22 Glaser, E., Hamasur, B., Norling, B. and Andersson, B. (1987) FEBS Lett. 223, 304–308.
23 Horak, A., Horak, H. and Packer, M. (1987) Biochim. Biophys. Acta 890, 302–309.
24 Norling, B., Hamasur, B. and Glaser, E. (1987) FEBS Lett. 223, 309–314.
25 Racker, E. and Horstmann, L.L. (1967) J. Biol. Chem. 242, 2547–2551.
26 Tzagoloff, A., MacLennan, D.H. and Byington, K.H. (1968) Biochemistry 7, 1695–1602.
28 Peterson, G.L. (1977) Anal. Biochem. 83, 346–356.
29 Penefsky, H.S., Pullman, M.E., Datta, A. and Racker, E. (1960) J. Biol. Chem. 235, 3330–3336.
30 Nordenbrand, K., Hundal, T., Carlsson, C., Sandri, G. and Ernster, L. (1977) in Bioenergetics of Membranes (Packer, L. et al., eds.), pp. 435–446, Elsevier/North-Holland, Amsterdam.
31 Sandri, G., Suranyi, E., Eriksson, L.E.G., Westman, J. and Ernster, L. (1983) Biochim. Biophys. Acta 723, 1–6.
32 Hundal, T., Norling, B. and Ernster, L. (1983) FEBS Lett. 162, 5–10.
33 Rusell, L.K., Kirkley, S.A., Kleyman, T.R. and Chan, H.P. (1976) Biochem. Biophys. Res. Commun. 73, 434–443.
34 Horak, H., Packer, M. and Horak, A. (1988) Biochim. Biophys. Acta 933, 389–392.
35 Glaser, E., Hamasur, B., Tourikas, C., Norling, B. and Andersson, B. (1989) Plant Physiol. Biochem., in press.