ON THE SUBUNIT COMPOSITION OF THE COUPLING FACTOR (ATPase) FROM *RHODOSPIRILLUM RUBRUM*

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1 . Introduction

Coupling factor proteins (F_1, CF_1, ATPase), purified to homogeneity from mitochondria, chloroplasts and aerobic bacteria can be dissociated in sodium dodecylsulfate (SDS) into subunits of different molecular weights. From all these sources, five or more subunits of different size have been detected [1–5].

This paper presents data, which demonstrate the existence of five different subunit sizes of polypeptides present in the coupling factor protein purified to apparent homogeneity from the photosynthetic bacterium *Rhodospirillum rubrum* [6,7]. This coupling factor shows a Ca^{2+}-dependent ATPase activity, which is stimulated during purification [6]. Evidence for partial dissociation of the enzyme as a result of treatment with LiCl is also presented.

2. Materials and methods

The coupling factor was isolated from *R. rubrum* chromatophores by use of methods described previously [6], and an additional purification step, chromatography on a column of DEAE Sephadex-A50 [7]. The enzyme appeared to be homogenous as judged by polyacrylamide gel-electrophoresis according to Davis [8] and by immunoelectrophoresis [7].

Dissociation into subunits was performed by overnight treatment at 37°C with 1% SDS plus 1% β-mercaptoethanol. Subunit composition was analyzed by SDS-polyacrylamide gel electrophoresis on calibrated gels as described by Weber and Osborn [9]. Reference proteins were: bovine serum albumin, trypsin, ovalbumin, ribonuclease, alcohol dehydrogenase and cytochrome *c*. After staining with Coomassie Blue, the destained gels were scanned in a Pye Unicam Spectrophotometer equipped with a gel cuvette.

ATPase activity was measured by methods previously described [6,10], and protein concentration was estimated by the Lowry method [11]. All reagents were of analytical grade.

3. Results and discussion

Five classes of subunits were detected after densitometric scanning of the gels (fig.1). The mobili-

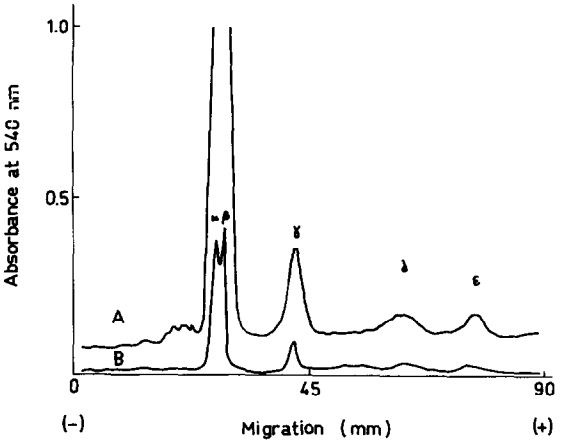

Fig.1. SDS-polyacrylamide gel-electrophoresis of purified coupling factor from *R. rubrum*. 100 μg (trace A) and 20 μg (trace B) ATPase was prepared and run as described in Materials and methods and in [9].

Table 1
Increase in total ATPase in three consecutive purification steps

Step	Specific ATPase activity	Total ATPase activity	Increase in total ATPase activity	
	μmol hr^{-1} mg^{-1}	μmol h^{-1}	relative to step 1	relative to step 2
1. Crude extract	0.4	133	1	–
2. 30–55% Ammonium sulfate fraction	3.6	359	2.7*	1
3. Sepharose 6B fractions (pooled)	80	1.419	10.7	4.0**

* Mean value for 3 different preparations is 2.2.
** Mean value for 4 different preparations is 4.3.
Measurements were performed as described in Materials and methods. For assay of ATPase 700 μg from step 1 was used; 600 μg from step 2; 17 μg from step 3.

ties corresponded to mol. wts of 54 000 (α), 50 000 (β), 32 000 (γ), 13 000 (∂), and 7500 (ϵ). These values are in close agreement with those reported for coupling factors isolated from other sources [1–5]. Detection of the two minor subunits (∂ and ϵ) required the analysis of a relatively large amount of enzyme, whereas resolution of the α- and β-subunits was obtained only with a much smaller amount.

The mol. wt of the entire ATPase molecule is about 350 000 [6], which is similar to ATPases from other organisms [12,13].

The Ca^{2+} ATPase activity of the *R. rubrum* coupling factor is stimulated several fold (increase in total ATPase) during purification (table 1). A low mol. wt ATPase inhibitor of still unknown nature and physiological role has been isolated from the crude coupling factor extract (to be published). The inhibitor may be a peptide constituting a sixth loosely associated component. The subunit structure of this ATPase complex from *R. rubrum* may thus be similar to that proposed by Brooks and Senior for F_1 from beef heart mitochondria [14] but different from that found for CF_1 from chloroplasts, where the inhibitor has been identified with the fifth firmly bound subunit (called the ϵ-subunit) [15]. If it is not properly activated, the ATPase activity of CF_1 is insignificant [16] compared to that of the coupling factors from mitochondria and *R. rubrum* chromatophores. The coupling factor from the photosynthetic bacterium *Rhodopseudomonas capsulata* has also extremely low ATPase activity [17]. Its subunit composition is still unknown.

In some cases it has been shown that the subunit pattern of the ATPase might depend on the method of solubilization [18,19]. LiCl has been used specifically to inactivate photophosphorylation, ATPase and ATP-linked transhydrogenase of *R. rubrum* chromatophores [20]. Restoration of photophosphorylation could, however, not be accomplished by addition of LiCl-extract to the treated membranes [21]. Since extraction of submitochondrial particles with salt solutions selectively solubilizes inactive subunits of F_1 from the membrane [22]. it was suggested that the LiCl might also cause a release of subunits of the ATPase from the chromatophore membrane [20]. By performing the LiCl-treatment in the presence of ATP Binder and Gromet-Elhanan could extract a component able to restore photophosphorylation [23]. As can be seen in fig.2, LiCl causes an inactivation of the ATPase in our purified coupling factor preparation. Although ATP protects the enzyme, this effect is quite limited. Under the conditions used by Binder et al. (2M LiCl

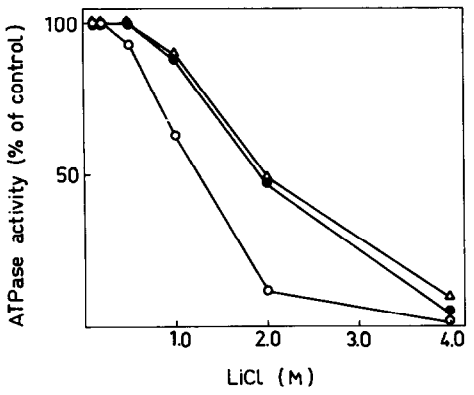

Fig.2. Effect of LiCl on the purified ATPase from *R. rubrum*. 0.032 mg of protein was preincubated with LiCl, and LiCl plus ATP for 10 min at 0°C in a total volume of 0.04 ml. This mixture was diluted to 1 ml during assay of ATPase, which was performed as described in Materials and methods. The partially purified ATPase preparation used had a specific activity of 240 mol P_i h^{-1} mg^{-1}. (○–○) no ATP; (●–●) 1 mM ATP; (△–△) 4 mM ATP.

plus 1–4 mM ATP) [20] about 50% of the enzymatic activity is lost. The sigmoidal type of inhibition (fig.2) might indicate that some type of structural rearrangements are taking place in the molecule, possibly a dissociation into subunits.

To test this hypothesis a sample of ATPase (43 μg) was preincubated for 10 min at 0°C with 2 M LiCl in the presence of 2 mM ATP in a total vol of 0.06 ml. After dilution with water to a volume of 0.2 ml this sample was layered on top of a sucrose density gradient (8–25%), which was centrifuged in a SW 56 Ti rotor at 39 000 rev/min for 20 hr. Reference gradients containing untreated ATPase and bovine serum albumine (50 μg) were run at the same time. Fluorimetric assay of the fractions [7] obtained after elution of the gradients, revealed that the ATPase treated with LiCl was partially converted to a species sedimenting to a position in the upper part of the gradient slightly above bovine serum albumin. Some of the treated enzyme was found in the lower part at the same position as the native ATPase ($S_{20,w}$ = 13; [6]). Analysis by polyacrylamide gel- electrophoresis [8] showed that the sample treated with LiCl produced two bands, one corresponding to the native enzyme (R_f = 0.14; 47% of the total stain), the other with considerably higher mobility (R_f = 0.76; 53% of the total stain). A band with a mobility indistinguishable from the latter was also found in aged or urea-treated preparations. Urea has been found to have an effect similar to that of LiCl on *R. rubrum* chromatophores [20] (an immunological characterization of products after urea treatment of the ATPase has been performed [7]). Thus, compared to the methods previously devised by us which yield a high mol. wt component active in ATPase as well as in recoupling [6,24], coupling factor extraction from *R. rubrum* chromatophores by LiCl or urea appears to result in a less well defined product, possibly containing inactive subunits.

The energy conservation system of *R. rubrum* chromatophores shows some significant differences when compared to most other systems from which coupling factors have been solubilized, especially with regard to its reactions involving PP_i [25,26]. However, the basic similarity, not only in function but also on the protein structural and substructural levels, between coupling factor ATPases isolated from eukaryotic and prokaryotic organisms is further underlined by these results with a photosynthetic bacterial system. In *R. rubrum* chromatophores, the reactions involving ATP, in contrast to those involving PP_i and utilizing a different membrane-bound enzyme [20,26], are sensitive to oligomycin. Thus, with respect both to coupling factor subunit characteristics and sensitivity to this inhibitor (the latter indicating that chromatophores may contain an additional protein similar to the mitochondrial oligomycin-sensitivity conferring protein, OSCP) the energy conservation apparatus of chromatophores shows a greater similarity to that of animal mitochondria than to that of plant chloroplasts.

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