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SEQUENTIAL REMOVAL AND RECONSTITUTION OF SUBUNITS β AND γ FROM A MEMBRANE-BOUND Fo·F$_1$-ATP SYNTHASE

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SUMMARY: The β and γ subunits of the Fo·F$_1$-ATP synthase complex of Rhodospirillum rubrum chromatophores were removed in two consecutive steps. The resulting depleted chromatophores lost all their ATP synthesizing activity but retained 70% of the light-induced proton uptake. ATP synthesis could be restored by reattachment of the isolated β and γ subunits together, but not of either one of them separately. These data suggest that the γ and β subunits are required for the operation of the chromatophore ATP synthase, but do not seem to participate in the light-induced proton uptake.

The coupling device of energy-transducing membranes, the Fo·F$_1$-ATP synthase, is composed of two distinct structures (1, 2). A soluble catalytic ATPase sector (F$_1$), which consists of five polypeptide subunits and a hydrophobic membrane sector (Fo), involved in the flux of protons across the membrane, which in chloroplasts and various bacterial systems consists of three subunits (3-6). The elucidation of the mechanism of action of the Fo·F$_1$-ATP synthase complex is dependent on a precise determination of the function of each of its individual subunits. A system allowing the sequential removal of single subunits, in their native active state, leaving a fully reconstitutable membrane lacking only the removed subunits, would be ideal for checking the specific role of each subunit both on and off the membrane. Such a system has bow been developed in R. rubrum chromatophores.

We have earlier shown that extraction of these chromatophores with LiCl removed completely the β-subunit of the RrF$_1$[1]-ATPase complex, leaving the other four subunits attached to the membrane (7). The resulting β-less chromatophores lost all their ATP synthesis and hydrolysis activities, but retained their capacity for light-induced proton uptake resulting in the formation of an electrochemical proton gradient (8, 9). Upon re-attachement of the purified β-subunit all the ATP-linked activities were fully restored (7).

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[1]Abbreviations: RrF$_1$ - the F$_1$ of R. rubrum; RrFo·F$_1$ - the Fo·F$_1$ of R. rubrum
SDS - sodium dodecyl sulfate; Mes - 2-(N-morpholino)ethane sulfonic acid;
Tricine-N-tris (hydroxymethyl)methylglycine

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In the present paper we describe a method for removal of an additional subunit, the γ-subunit, from the β-less R. rubrum chromatophores. The functions of both the β and γ-subunits have been investigated by testing various activities of the depleted membranes before and after reattachment of the removed subunits.

MATERIALS AND METHODS

R. rubrum cells (strain S1) were grown as previously described (7), and coupled chromatophores were prepared according to published procedures (8-10). The β-subunit of RrF_1 and β-less chromatophores were obtained by treating choupled chromatophores with 2 M LiCl in the presence of 4 mM ATP as previously described (9), and the isolated β-subunit was purified as outlined by Philosoph et al. (7).

The method adopted for removal of the γ-subunit from the β-less chromatophores involved extraction with 2 M LiBr in a medium containing 0.05 M Tricine-NaOH, pH 7.6; 0.25 M sucrose; 4 mM ATP and 0.5 mg bacteriochlorophyll/ml. The suspension was stirred for 1 hr at 4° and then centrifuged, washed and resuspended according to the procedure used for LiCl extraction (8). The protein present in the LiBr-extract was partially purified by $(NH_4)_2SO_4$ fractionation. The reconstituting activity precipitated at 30% $(NH_4)_2SO_4$ saturation.

Photophosphorylation was assayed as outlined by Philosoph et al. (7). Light induced proton uptake was measured as described by Gromet-Elhanan and Leiser. (11). SDS-polyacrylamide gel electrophoresis was performed on 10% acrylamide tube gels (12). Samples were boiled for 2 min in the presence of 1% SDS, 0.1% β-mercaptoethanol and 5% glycerol prior to application. Bacteriochlorophyll was determined using the absorbance coefficient in vivo given by Clayton (13) and protein was measured according to Lowry et al. (14).

RESULTS

As illustrated in Table 1 extraction of R. rubrum chromatophores with LiCl resulted in complete loss of photophosphorylation, but not of the light-induced proton uptake. Even after a further extraction with LiBr about 70% of the proton uptake capacity was retained by the treated chromatophores. Differences between these two types of treated chromatophores were revealed in reconstitution studies. In the LiCl-treated chromatophores, which lost only their β-subunit (7), photophosphorylation could be completely restored after reconstitution of the missing β-subunit, whereas in the LiCl-LiBr treated chromatophores there was no restoration of photophosphorylation when either the purified β-subunit or the protein present in the LiBr-extract were added. ATP synthesis could, however, be restored upon addition of both the β-subunit and the LiBr-extract, but only to about 22% (Table 1). This percentage did not increase even when the depleted chromatophores were incubated with larger amounts of the protein present in the LiBr-extract (15).

The additional protein removed by the LiBr extraction consisted, even without any extensive purification, mainly of one polypeptide, which electrophoresed on SDS-

TABLE 1

Effect of various treatments of R. rubrum chromatophores on their light-induced proton uptake and ATP synthesis before and after reconstitution

Activity tested	Protein present during reconstitution	Treatment of chromatophores	
		LiCl	LiCl followed by LiBr
		% of control	
Proton uptake	none	93	70
ATP synthesis	none	2	1
ATP synthesis	purified β-subunit	90	2
ATP synthesis	LiBr-extract	2	1
ATP synthesis	LiBr-extract and purified β-subunit	91	22

Extraction of coupled chromatophores with LiCl and LiBr was carried out as described under Materials and Methods. Reconstitution was obtained by incubating the treated chromatophore preparations (10 μg bacteriochlorophyll) with the indicated soluble preparations (0.2 mg of protein) at 35° for 30 min in the medium described by Gromet-Elhanan at pH 7.5 (8). Photophosphorylation was assayed immediately after the reconstitution. Control activity in coupled chromatophores was 2.2 μeq. protons taken up/mg bacteriochlorophyll and 815 μmol ATP synthesized/h per mg bacteriochlorophyll.

acrylamide gels with an apparent molecular weight identical to that of the γ-subunit of RrF1 (Fig. 2).

Reconstitution of ATP synthesizing activity in both types of the depleted R. rubrum chromatophores was carried out at pH 7.5 (Table 1), since at this pH complete reconstitution of this activity was obtained in the β-less chromatophores upon addition of the purified β-subunit (7). Reconstitution of soluble ATPase activity from a mixture of isolated α,β

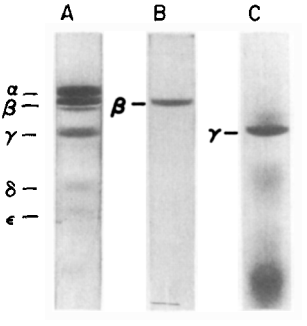

Fig. 1: SDS-polyacrylamide gel electrophoresis of RrF_1 and its isolated subunits

30 μg of protein was applied on each SDS-gel. A - Purified RrF_1; B - purified β-subunit of RrF_1; C - a 30% $(NH_4)_2SO_4$ fraction of the LiBr-extract of β-less chromatophores.

TABLE 2

Effect of pH during reconstitution on the restoration of ATP synthesizing activity in depleted chromatophores

Reconstitution system tested		pH during reconstitution		
depleted chromatophores	soluble subunits	8.0	7.2	6.2
		μmol ATP formed/h per mg Bchl		
β-less	β	810	800	840
β,γ-less	β	25	20	17
β,γ-less	γ	4	-	3
β,γ-less	β+γ	180	330	520

Reconstitution was obtained by incubating the depleted chromatophores (10 μg Bchl) either with the purified β-subunit (150 μg protein) and/or with the γ-subunit present in the 30% $(NH)_2SO_4$ fraction of the LiBr-extract (75 μg protein) under the condition described in Table 1, except that the pH was varied, as indicated by using 10 mM Mes-NaOH for pH 6.2 and 7.2 and 10 mM Tricine-NaOH for pH 8.0. Photophosphorylation was assayed as outlined by Philosoph et al. (7), but with 80 mM Tricine-NaOH, pH 8.0. Bchl = bacteriochlorophyll.

and γ subunits was recently reported by two groups (16, 17). Both groups found that the reconstitution was optimal when it was carried out around pH 6.0 rather than 8.0, even though the ATPase assay was always run at pH 8.0. If this low pH is required for maintaining an optimal reconstitution capacity of mixtures containing a soluble γ-subunit, it might also be required for reconstitution of the ATP synthesizing activity in chromatophores depleted of γ upon addition of this soluble subunit. Reconstitution of the LiCl-treated (β-less) and the LiCl-LiBr-treated (β, γ-less) chromatophores with the isolated subunits was therefore tested at a pH of 6.2, 7.2 and 8.0.

The results summarized in Table 2 indicate that reconstitution of photophosphorylation in the presence of the γ-subunit is indeed optimal at a low pH. Whereas photophosphorylation was restored to the same maximal extent when the β-less chromatophores were reconstituted with the purified β-subunit at pH 6.2, 7.2 or 8.0, a completely different pattern was observed with the β,γ-less chromatophores. In the presence of both β and γ a three fold increase of the restored photophosphorylation occurred when the pH during reconstitution was lowered from 8.0 to 6.2. Thus, after reconstituting the β, γ-less chromatophores with both missing subunits at pH 6.2, more than 60% of the ATP synthesizing capacity of fully coupled chromatophores was restored. In the presence of either β or γ alone no photophosphorylation activity could be reconstituted in the β, γ-less chromatophores even at pH 6.2. With all these systems photophosphorylation was assayed at pH 8.0 (Table 2).

DISCUSSION

The present experiments provide a new approach to the study of the structure and function of the Fo·F_1-ATP synthase, which involves a stepwise removal of individual subunit from this complex at its membrane-bound state. Two subunits, β and γ, were removed from coupled chromatophores of the photosynthetic bacterium R. rubrum by subjecting them to two consecutive extractions, first with LiCl followed by LiBr. Extraction with LiCl has earlier been shown to remove completely one single subunit, the β subunit from the RrFo·F_1 complex (7), thus enabling us to study the structure and function of the solubilized β-subunit, the remaining β-less chromatophores and their possible reconstitution.

The β-less chromatophores lost all their ATP synthesis and hydrolysis activities (7-9), but retained their light-induced proton uptake (9, see also Table 1). The other four RrF_1-subunits remained attached to these β-less chromatophores. This was shown in two ways: a. by applying the chloroform method for removal of F_1 (18) on the β-less chromatophores, a four subunit RrF_1-complex lacking β was released (7) and b. upon reattachment of the missing solubilized β-subunit to the β-less chromatophores their ATP synthesis and hydrolysis activities were completely restored (7, 9). The solubilized β-subunit was purified to homogenity and found to be composed of one single polypeptide (7, see also Fig. 1), which had by itself no ATPase activity, although it could restore all ATP-linked activities to the β-less chromatophores (7, see also Tables 1 and 2). Moreover, antibodies prepared against the purified β inhibited all ATP-linked activities in coupled or reconstituted R. rubrum chromatophores as well as in solubilized RrFo·F_1 and RrF_1 preparations (19). It was therefore concluded that the β-subunit is absolutely necessary for catalysis of ATP-linked activities, but does not participate in the translocation of protons across the membrane.

Removal of an additional subunit, the γ subunit, from the β-less chromatophores has now been achieved by their extraction with LiBr (Fig. 1). The resulting β, γ-less chromatophores still retained about 70% of their light-induced proton uptake, but their photophosphorylating activity could not be restored by reconstitution with either β or γ alone (Tables1 and 2). It could be restored upon reconstitution of both missing subunits, provided that the pH during reconstitution was around 6.2 (Table 2). Further characterization of the γ subunit and the β, γ-less chromatophores is now in progress. However, even from the above results it is already obvious that the solubilized β and γ subunits have different properties. Unlike β, γ seems to require a low pH in order to maintain its capacity for reconstituting either the soluble ATPase activity (16, 17) or the membrane-

bound ATP synthesizing activity (Table 2). It is interesting to note in this respect that Kozlov et al.(20) have recently reported on the removal of subunit a from submitochondrial particles with a consequent loss of their ATPase activity. In this case, the capacity of the isolated a to restore the membrane-bound oligomycin-sensitive ATPase was markedly enhanced by its maintenance in a reduced form, a property not shared by either β or γ. Moreover, the reconstituting capacity of α was completely blocked by 1 mM ATP (20), whereas the reconstituting capacity of β was completely dependent on the presence of 1-2 mM ATP (7, 9). Experiments of this type can thus supply useful information connecting specific properties of the isolated single subunits with their function.

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