Short Communication

F_1-ATPase from *Rhodopseudomonas blastica*

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The genes for the catalytic part of the F_0F_1-ATPase from two different phototrophic bacteria, *Rhodospirillum rubrum*[1] and *Rhodopseudomonas blastica*,[2] have been sequenced. The sequence of the genes for the F_0-moiety of the former bacterium is also known.[3] The *atp*-operon of *R. rubrum* encodes the five subunits found in preparations of the enzyme, i.e. α, β, γ, δ, ε. The *atp*-operon of *Rps. blastica* also contains a sixth gene, termed *X*. The protein has not previously been prepared from *Rps. blastica*. A pure preparation of the enzyme is described in this communication. In addition, the preparation is compared with purified F_1-ATPase from *R. rubrum*.

Experimental

Rps. blastica[4] and *R. rubrum*[5] cells were grown and harvested as described elsewhere. Chromatophores were prepared and the bacteriochlorophyll content was estimated mainly as has been described earlier.[4,6-8]

The F_1-ATPase from *R. rubrum* was prepared by a modification[9] of a previously described method for rat liver mitochondrial F_1-ATPase.[10] It has been found by *N*-terminal analysis that the polypeptides of this preparation were identical with the gene products of the *atp*-operon.[†] The same method[9] was employed for the preparation of the F_1-ATPase from *Rps. blastica*.

SDS gel electrophoresis was carried out as described in Ref. 11. The ATP hydrolytic activity was followed by a colorimetric method[12,13] and protein was estimated by the Bradford method[14] (Bio-Rad).

Results and discussion

Fig. 1 shows a silver-stained SDS electrophoresis gel with the F_1-preparations from *R. rubrum* and *Rps. blastica*. In Table 1, the molecular weights determined from the gel are compared with those for the *R. rubrum* enzyme given in Ref. 1 and the *Rps. blastica* F_1 calculated from the genes[2] by using an average of 107 Da per amino acid. Six bands were routinely obtained for the *Rps. blastica* enzyme. One

[†]Walker, J. E., Falk, G. and Strid, Å. *Unpublished results.*

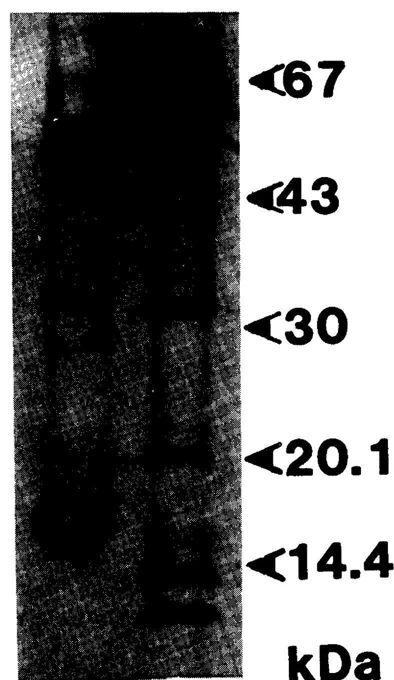

Fig. 1. A silver-stained SDS polyacrylamide electrophoresis gel showing the F_1-ATPase preparations from *R. rubrum* (left lane) and *Rps. blastica* (right lane). The molecular weight standards are indicted by arrows.

Table 1. The molecular weights of the polypeptides of the preparations of this study compared with the molecular weights calculated from the genes.

Molecular weights/kDa			
Rps. blastica		*R. rubrum*	
Calculated from Ref. 2	This study	From Ref. 1	This study
55.2 (α)	59	55.0 (α)	60
51.3 (β)	53	50.8 (β)	55
31.0 (γ)	32	32.4 (γ)	33
28.8 (*X*)	20.4	19.5 (δ)	18
19.5 (δ)	13.9	14.3 (ε)	16
13.9 (ε)	12.5		

Table 2. Comparison between the ATPase activities of different preparations from *Rps. blastica* and *R. rubrum.*[a]

Additions	*Rps. blastica*	*R. rubrum*
F_1		
5 mM Ca^{2+}	2.1	3.8
5 mM Ca^{2+} + 50 mM HCO_3^-	3.8	4.1
5 mM Ca^{2+} + 50 mM HCO_3^- + 20 μM DES[b]	2.3	0.6
1.5 mM Mg^{2+}	0.4	0
1.5 mM Mg^{2+} + 50 mM HCO_3^-	0.8	0.2
1.5 mM Mg^{2+} + 50 mM HCO_3^- + 20 μM DES	1.2	1.4
Chromatophores		
5 mM Ca^{2+}	0.16	1.4
5 mM Ca^{2+} + 2 μM FCCP	0.19	1.0
1.5 mM Mg^{2+}	0.32	1.1
1.5 mM Mg^{2+} + 2 μM FCCP	0.45	3.9

[a]2.5 mM ATP was present in all experiments. The hydrolytic activities are expressed as μmol ATP (mg protein)$^{-1}$ min^{-1} and μmol ATP (μmol bacteriochlorophyll)$^{-1}$ min^{-1} for solubilized F_1 and the chromatophores, respectively. [b]DES = diethylstilbestrol.

of these bands might correspond to the gene product of the *X* gene.

A comparison between some of the properties of the ATP hydrolytic activity of F_1-ATPase purified from *Rps. blastica* and from *R. rubrum,* and between the properties of the ATPase activity of the chromatophores from the same organism is shown in Table 2. It is evident that both preparations of F_1 show the highest activity with Ca^{2+} as the divalent cation. HCO_3^- is an activator in both cases, and for both preparations, diethylstilbestrol inhibits Ca^{2+}-promoted activity but increases Mg-ATPase activity. Mg-ATP hydrolysis in chromatophores from *R. rubrum* is strongly stimulated by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). On the other hand, the same preparation from *Rps. blastica* is only slightly stimulated. This probably reflects the fact the intracellular membranes continuous with the plasma membrane in *Rps. blastica* are lamellar, whereas the corresponding membranes in *R. rubrum* are spherical or tubular. Thus, it is likely that the chromatophore preparations from the former bacterium contain fewer closed vesicles than preparations from the latter.

In accordance with the observations with *R. rubrum,* oligomycin and venturicidin inhibit ATP hydrolysis in chromatophores from *Rps. blastica* (not shown).

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