SOLUBILIZATION OF AN OLIGOMYCIN-SENSITIVE ATPase COMPLEX FROM *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES AND ITS INHIBITION BY VARIOUS ANTIBIOTICS

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1. Introduction

F_0–F_1 ATPase complexes play an important role in oxidative phosphorylation, photophosphorylation and ATP-driven functions. The complete ATPase complexes are mainly composed of two distinct structural units. The one part, the so-called F_1 component, is located at the inner surface of the membrane. It has been purified to homogeneity from a variety of eucaryotes and procaryotes and exhibits ATPase activity [1]. The other part, often referred to as F_0, is deeply inserted into the membrane and directs the proton flow through the membrane. These two major parts are thought to be connected by a protein complex, that confers oligomycin sensitivity from the F_0 component to the F_1 component. In some cases the connective protein complex appears to have the structure of a stalk. The oligomycin-sensitivity conferring protein (OSCP-protein) and the ATPase inhibitor are apparently not obligatory for all F_0–F_1 ATPase complexes. Very little is known about the membrane counterpart of the chloroplast factor CF_1 and its role in photophosphorylation [2]. Investigation of ATPase complexes of a facultative photosynthetic bacterium could therefore lead as well to some general conclusions on the function of photophosphorylation and oxidative phosphorylation.

The present investigation has been directed towards the isolation of the F_0–F_1 ATPase complex from chromatophores of *Rhodospirillum rubrum* and its characterization by different antibiotics.

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2. Materials and methods

Citreoviridin was purchased from Chambrian Chemicals (Croydon, England). Oligomycin was obtained from Serva (Heidelberg, FRG). All other antibiotics were generous gifts from the following companies: peliomycin from Bristol-Myers (New York), efrapeptin and rutamycin from Eli Lilly (Indianapolis). Botrycidin was kindly donated by W. Keller-Schierlein (Zürich). Phosphatidylcholine was purchased from Sigma (St Louis). All other chemicals were products from Merck (Darmstadt, FRG).

ATPase activity was measured in 50 mM Tris–HCl, pH 8.0, in the presence of 1 mM $CaCl_2$ and 1 mM ATP at 37°C by determining the liberated orthophosphate as in [3]. Protein concentration was measured as in [4].

All inhibitors were dissolved in ethanol. Before adding the substrate, the enzyme-inhibitor solution was preincubated for 20 min at 0°C. Residual activity was measured as described before.

Gel electrophoresis was carried out on calibrated 12% (w/v) polyacrylamide gels in the presence of 0.2% (w/v) SDS as in [5]. Before application to the gel the ATPase complex was dissociated into subunits by treatment with 2% (w/v) SDS, 2% (v/v) β-mercapto-

ethanol and 0.1 M potassium phosphate buffer, pH 7.2, at 100°C for 5 min. The reference proteins were: RNA polymerase, bovine serum albumin, trypsin inhibitor and cytochrome *c*. After staining with Coomassie brilliant blue R 250 the gels were scanned in a Gilford spectrophotometer 2400 using a gel cuvette.

R. rubrum FR 1 cells were grown photoheterotrophically in a 15 liter fermenter at 30°C in R8ÄH-medium [6]. Chromatophores were prepared by ultrasonication as in [7].

Solubilization of the ATPase complex was carried out essentially as in [8]. Chromatophores (2.5 mg/ml) were suspended in a buffer containing 20% (v/v) glycerol, 50 mM Tris–HCl, pH 8.0, 5 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.01% (w/v) dithiothreitol. Triton X-100 was added to final conc. 0.25% (v/v). This value corresponds to a protein–detergent ratio of approx. 1:1. After incubation for 30 min at 0°C the solution was centrifuged for 90 min at 200 000 × *g*. The supernatant, which contained the ATPase complex, was concentrated either by ultrafiltration or by dialysis against the above-used buffer containing 50% (w/v) sucrose, and further purified by centrifugation through a linear glycerol density gradient (5–20%, w/v) for 12 h at 130 000 × *g*. Alternatively, purification could be achieved by gel filtration on a Sepharose CL-4B column (2.5 × 70 cm). Elution was carried out with a buffer containing 50 mM Tris–HCl, pH 8.0, 5% (v/v) glycerol, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.01% (w/v) dithiothreitol and 0.1% (v/v) Triton X-100.

3. Results and discussion

An ATPase complex could be solubilized from chromatophores of *R. rubrum* by treatment with Triton X-100. At final conc. 0.25% (v/v) Triton X-100 in the extraction buffer the ATPase complex was preferentially released. Higher concentrations of detergent led to the release of additional proteins and also inactivated the ATPase.

F_0–F_1 ATPase complexes, sensitive to oligomycin or related antibiotics, have been first solubilized from beef heart and yeast mitochondria by treatment with Triton X-100, cholate or related detergents [9]. Purification is often complicated by denaturation of the complex. Only one highly stable ATPase complex has been described [10]. Compared with these ATPases the enzyme of *R. rubrum* is relatively stable in the above-mentioned buffer and could be further stabilized by adding phosphatidylcholine at final conc. approx. 5 mg/ml [11].

Purification of the ATPase complex could be achieved in two alternative ways; as in section 2. Gel filtration on a Sepharose CL-4B column yields a preparation with a specific activity of about 1 μmol substrate/min·mg protein (table 1). This is about 2.5-times the specific activity of the same ATPase complex purified by glycerol gradient centrifugation.

SDS–polyacrylamide gel electrophoresis reveals the presence of at least 13 different subunits with mol. wt 57 000–6500. The location of the three major subunits of the F_1 ATPase (α, β and γ) is indicated for comparison [12].

The catalytic activity of the ATPase complex depends on Ca^{2+}- or Mg^{2+}-ions in the same manner as that of the membrane-bound enzyme, whereas the water-soluble F_1 ATPase hydrolyzes only Ca^{2+}-ATP [13].

The effects of various antibiotics on the membrane-bound ATPase, on the detergent-dispersed F_0–F_1 ATPase complex and on the soluble F_1 ATPase from *R. rubrum* are shown in table 2.

Oligomycin and related compounds appear to inhibit the specific transfer of protons through the

Table 1
Properties of ATPase preparations during purification

Fraction	Total prot. (mg)	Spec. act. (μmol P_i/min·mg)	Purification factor
Chromatophores	112.0	0.088	–
Triton extract	39.6	0.25	2.8
Glycerol gradient	5.1	0.40	4.5
Sepharose CL-4B	2.9	1.00	11.4

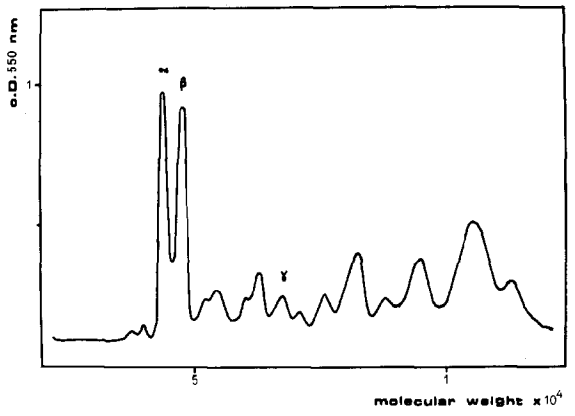

Fig.1. SDS polyacrylamide gel electrophoresis of the Triton X-100 solubilized ATPase complex from *R. rubrum*. Protein 100 μg was prepared as described in section 2.

F_0 part of the membrane, as could be shown for mitochondrial systems and for *R. rubrum* [14].

Oligomycin at a concentration of 20 μg/ml causes an approx. 50% inhibition of the F_0–F_1 ATPase activity. In the membrane-bound state the enzyme is about 70% inhibited by the same concentration of oligomycin. Practically no inhibition of the isolated F_1 ATPase could be observed (table 2).

Somewhat smaller inhibition values are obtained for oligomycin-related compounds such as rutamycin, peliomycin, and botrycidin. Our data indicate that, at least in the case of *R. rubrum*, botrycidin is an inhibitor best suited for distinguishing between F_1 and F_0–F_1, because it has almost no effect on F_1. It has been reported earlier that oligomycin does not affect photophosphorylation in chloroplasts, whereas it inhibits the ATP-synthase systems of bacterial photosynthetic organisms. Most non-photosynthetic procaryotes are not inhibited by oligomycin [15]. Our data show that coupling factors of chromatophores from *R. rubrum* are in this respect more related to mitochondrial systems than to those of chloroplasts.

Citreoviridin as well as efrapeptin are potential inhibitors of the energy-conserving systems [15]. They completely inhibit the soluble states (table 2). With respect to efrapeptin these results agree with [16]. In contrast to our results, other bacterial systems, e.g., the ATPase of *Escherichia coli* require much higher efrapeptin concentrations for inhibition [15]. In view of the very low concentrations of efrapeptin necessary for complete blocking of ATP hydrolysis in *R. rubrum* it seems likely that the binding site for efrapeptin may be close to the catalytic site, the site II [17]. As shown in table 2 ATPase of *R. rubrum* is inhibited by citreoviridin in

Table 2
Inhibitory effect of various antibiotics on ATPase preparations from chromatophores of *Rhodospirillum rubrum*

	Membrane-bound ATPase		F_0–F_1 ATPase complex		F_1 ATPase	
Inhibitor	Inhibitor (μg/ml)	Inhibition (%)	Inhibitor (μg/ml)	Inhibition (%)	Inhibitor (μg/ml)	Inhibition (%)
Oligomycin (A:B:C, 60:30:10)	20	72	20	56	20	6
Peliomycin ('Oligomycin B')	20	69.9	20	44	20	3.4
Rutamycin (= Oligomycin D)	20	64.2	20	41	20	16
Botrycidin	20	61.1	20	44	20	1.4
Efrapeptin	0.3	100	0.3	100	0.3	100
Citreoviridin	20	82.4	20	100	20	100

the membrane-bound, in the detergent-dispersed as well as in the water-soluble state. But in contrast to efrapeptin much higher inhibitor concentrations are needed to obtain the same amount of inhibition. Effects of citreoviridin on bacterial systems have not been reported earlier.

Note

When preparing this manuscript an independent investigation on the preparation of the F_0–F_1 complex of *R. rubrum* was published: Oren, R. and Gromet-Elhanan, Z. (1977) FEBS Lett. 79, 147–150.

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