On the Oligomycin-Sensitivity and Subunit Composition of the ATPase Complex from *Rhodospirillum rubrum*

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Two alternative procedures for isolation of the oligomycin-sensitive ATPase complex (E. C. 3.6.1.3) from *R. rubrum* chromatophores are compared with respect to ease, rapidity, and yield.

The inhibitory effect of oligomycin on the membrane-bound Ca^{2+}-ATPase activity is increased during storage of the chromatophores, whereas the effect of oligomycin on the membrane-bound Mg^{2+}-ATPase activity does not change within a week.

Oligomycin-sensitivity of the solubilized ATPase complex depends on the isolation procedure.

The enzyme complex consists of at least nine different polypeptides with the apparent molecular weights of (1) 67,000, (2) 60,000, (3) 56,000, (4) 35,000, (5) 22,000, (6) 20,000, (7) 17,000, (8) 12,000, (9) 11,000. The polypeptides 2–4, 7, and 8 represent subunits of coupling factor 1.

Introduction

Adenosine triphosphatase (ATPase) complexes have been solubilized from mitochondria [1, 2], and bacteria [3–5]. These ATPases are composed of two structural parts [6]. One of them, the water soluble coupling factor 1 (F_1), catalyses ATP hydrolysis. The second part, often referred to as F_0, is buried in the hydrophobic interior of the membrane and lacks ATPase activity. This component catalyses specific hydrogen ion transport through membranes, which is sensitive to energy transfer inhibitors like oligomycin, rutamycin or N,N'-dicyclohexylcarbodiimide (DCCD) [3, 7, 8]. In some instances the subunit composition of the F_0 component is known [9, 10].

The water soluble entity, F_1, of the ATPase has been solubilized from chromatophores from a number of photosynthetic bacteria [11–14] and purified to varying degrees. The subunit composition of the enzyme from *R. rubrum* has been determined [15, 16] to consist of five different subunits which are similar to those of ATPases from other organisms.

Recently, the isolation and some immunochemical properties of an oligomycin-sensitive ATPase from *R. rubrum* was reported [17–19]. In this paper we present data on the subunit composition of the oligomycin-sensitive ATPase complex from *R. rubrum*, which are consistent with published data on ATPase complexes from other sources [2–4]. We also describe the effect of oligomycin on different ATPase preparations.

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Materials and Methods

R. rubrum cells (van Niel strain S1) were grown and harvested and chromatophores were prepared according to methods earlier described [20, 21].

The oligomycin-sensitive ATPase complex was solubilized and purified by two alternative procedures:

a) Prewashing of chromatophores with sodium cholate and subsequent extraction of the ATPase complex by Triton X-100.

Chromatophore membranes (1 g wet weight) were suspended in 10 ml 50 mM Tris-Cl, pH 8.0, containing 150 mM KCl and sodium cholate at a final concentration of 1% according to Yoshida *et al.* [22]. After incubation at 4 °C for 45 min the suspension was centrifuged at 150,000 x *g* for 45 min. The resulting sediment (treated chromatophores) was resuspended in 10 ml 50 mM Tris-Cl, pH 8.0, containing 25 mM sucrose, 150 mM KCl, and 0.2% Triton X-100 at 4 °C for 30 min. After recentrifugation of the suspension at 150,000 x *g* for 1.5 h an oligomycin-sensitive ATPase activity appeared in the resulting supernatant.

b) Solubilization of the ATPase complex from untreated chromatophores by Triton X-100 and further purification of the crude enzyme by glycerol-densitiy-gradient centrifugation according to Schneider *et al.* [18].

Dissociation into subunits was performed at 100 °C for 1 min in the presence of 2% sodium dodecyl sulfate (SDS) and 5% mercapto-ethanol. SDS-disc electrophoresis was carried out in 12% polyacrylamide gels containing 0.1% SDS according to Laemmli [23].

The samples contained 15 µg of protein and were run at 2 mA per gel with bromophenol blue as tracking dye. For molecular weight determinations calibration gels with bovine serum albumin, lactate dehydrogenase, and cytochrom *c* as reference proteins were used. Gels were stained with 0.25% (w/v) Coomassie brilliant blue G 250 for 1.5 h, destained in methanol : acetic acid : water (2 : 3 : 35), and scanned in a Gilford spectrophotometer model 2400 at 550 nm.

ATPase activity was measured by using the glass electrode technique described by Nishimura *et al.* [24]. 2.5 ml reaction medium contained 1 mM Tris-Cl, pH 7.9 and 4 mM $CaCl_2$ or 1 mM $MgCl_2$. Where mentioned, oligomycin dissolved in ethanol was added before the pH of the reaction medium was adjusted to 7.90 with diluted KOH or HCl. Control activities were measured under same conditions without inhibitor but with adequate amount of ethanol. After incubation for 3 min at 24 °C the reaction was started by addition of 4 mM ATP solution, pH 7.90.

Protein concentration was estimated by the Lowry method [25].

Results

The various steps and yields of our procedures are summarized in Table I. Compared to the membrane-bound ATPase the specific activities of the solubilized complexes increased 2 – 3 fold. Prepared by each of these methods the enzyme appeared to be at least 90% pure as judged by staining of protein bands in polyacrylamide gels (data not shown). There are, however, still both bacteriochlorophyll (BChl) and carotenoids associated with the complex (0.02 mg BChl/mg protein). This appears to be true also for the ATPase complex decribed in ref. 17, where its activity is given as units/mg BChl.

Table I. Preparation of the oligomycin-sensitive ATPase complex from *R. rubrum* chromatophores. Specific ATPase activity was measured in presence of 4 mM Ca-ATP^{2-} as described in Materials and Methods. One unit (U) is defined as the number of µmol of inorganic phosphate liberated during 1 min at 24 °C. For designation of fractions see Materials and Methods.

Fraction	Total protein [mg]	Specific activity [U/mg]	Total activity [U]
chromatophores	100.0	0.11	11.0
Triton X-100 extract of treated chromatophores	13.8	0.32	4.4
Triton X-100 extract of untreated chromatophores	18.6	0.29	5.3
eluate of glycerol-density-gradient	6.4	0.39	2.5

Subunit composition of the oligomycin-sensitive ATPase complex from R. rubrum

The ATPase complex prepared from treated chromatophores was analyzed for its subunit composition. Nine different protein bands were detected after densitometric scanning of the SDS gels (Fig. 1). The mobilities correspond to molecular weights given in Table II. In addition to the α, β, γ, δ, and ε-subunits of coupling factor 1 (15, 16) four polypeptides with apparent molecular weights of 67,000, 22,000, 20,000, and 11,000 are clearly distinguished. Protein band 1 (67,000) probably represents trace amounts of contaminants. The residual three bands may be components of the F_0 moiety. However, a better evaluation of the protein bands in the 11,000 molecular weight region is required. This region may include additional low

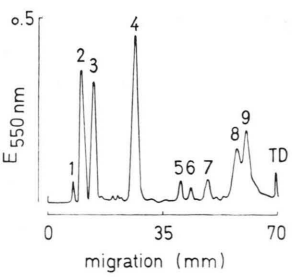

Fig. 1. Sodium dodecyl sulfate disc electrophoresis of the oligomycin-sensitive ATPase complex from *R. rubrum* chromatophores. 15 µg of ATPase complex solubilized from treated chromatophores were prepared and run as described in Materials and Methods. TD, tracking dye.

Table II. Molecular weights of subunit proteins of *R. rubrum* ATPase. The molecular weights were calculated from the mobilities of the proteins relative to that of bromophenyl blue in 12% polyacrylamide gels containing 0.1% SDS. The numbers of protein bands correspond to those shown in the densitometric trace of the gel in Fig. 1. Protein bands 2–4, 7, and 8 represent subunits of coupling factor 1.

Protein band	Oligomycin-sensitive ATPase complex [mol wt]	F_1ATPase
1	67,000	(–)
2	60,000	(α)
3	56,000	(β)
4	35,000	(γ)
5	22,000	(–)
6	20,000	(–)
7	17,000	(δ)
8	12,000	(ε)
9	11,000	(–)

molecular weight polypeptides. The significant staining intensity of protein band 4 (Fig. 1) corresponding to the γ-subunit of F_1 (Table II) is consistent with a chloroform-preparation of the F_1-ATPase [16].

Effects of oligomycin on different ATPase preparations

Both the membrane-bound Mg^{2+}- and Ca^{2+}-ATPase activities are sensitive to oligomycin [26]. As can be seen from Table III the ATPase activity in freshly prepared chromatophores is more inhibited by oligomycin (10 µg per ml) in the presence of Mg^{2+} than of Ca^{2+}. Inhibition is 90% and 26% of control, respectively. Similar results have previously been reported by our group [26] and recently also by Oren and Gromet-Elhanan [17]. Storage of the chromatophores for 7 days at 0 °C in 0.1 M glycylglycine buffer, pH 8.0, increases the oligomycin sensitivity of the Ca^{2+}-ATPase about 2.5 times. However, the effect of inhibitor on the Mg^{2+}-ATPase does not change within a week.

Table III. Effect of oligomycin on membrane-bound ATPase activities of *R. rubrum* chromatophores. Chromatophores (40 µg bacteriochlorophyll) were preincubated for 3 min with 25 µg oligomycin in 2.5 ml of reaction mixture and ATPase assay was performed as described in Materials and Methods.

Fraction	%-Inhibition of	
	Mg^{2+}-ATPase	Ca^{2+}-ATPase
freshly prepared chromatophores	90	26
aged chromatophores *	90	69

* Chromatophore preparation stored for 7 days at 0 °C.

Oligomycin-sensitivity of the solubilized ATPase complex depends on the isolation procedure. Prewashing of chromatophores with sodium cholate scarcely solubilized ATPase activity, but it removed other membrane components. Subsequent extraction of the membranes by Triton X-100 yields a nearly pure ATPase complex that is activated by both Ca^{2+} and Mg^{2+}. Both ATPase activities are nearly completely inhibited by oligomycin, as is shown in Fig. 2. Half maximum inhibition is achieved at about 2 µg oligomycin per ml (Fig. 2). A crude ATPase complex, showing the same oligomycin sensitivity, has been solubilized from untreated chromatophores by Triton X-100. Further purification by glycerol-densitiy-gradient centrifugation leads to a nearly pure enzyme. However, in presence of Ca^{2+} this complex shows only a maximum inhibition by oligomycin of 50% (Fig. 2). This agrees with the data obtained by Oren and Gromet-Elhanan [17] who used a similar preparation procedure.

The freshly isolated complexes from treated chromatophores are also sensitive to N,N-dicyclohexylcarbodiimide (DCCD) with a 50% inhibition of both Mg^{2+}- and Ca^{2+}-ATPase activities at 2–4 µg DCCD per ml (data not shown). The inhibitor sen-

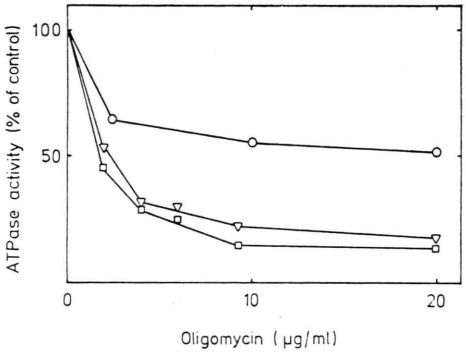

Fig. 2. Effect of oligomycin on F_0-F_1ATPase preparations from *R. rubrum* chromatophores. The reaction mixtures contained 50 µg of protein and Ca-ATP^{2-} and Mg-ATP^{2-} as described in Materials and Methods. (▽–▽) Ca^{2+}-ATPase activity of the complex extracted from treated chromatophores, (□–□) Mg^{2+}-ATPase activity of the complex extracted from treated chromatophores, and (○–○) Ca^{2+}-ATPase activity of the complex extracted from untreated chromatophores and further purified by glycerol-density-gradient centrifugation.

sitivity of the purified ATPase complexes is preserved for about 1 – 2 days.

Discussion

The oligomycin-sensitive ATPase complex from *R. rubrum* chromatophores plays a dominant role in the terminal reactions of photophosphorylation [26]. Its solubilization from cholate washed chromatophores by Triton X-100 is superior for the present purposes to the procedure starting with untreated chromatophores with respect to ease, rapidity and yield.

Oligomycin inhibition of the membrane-bound Ca^{2+}-ATPase depends on the age of the chromatophores. Increase in oligomycin-sensitivity may be correlated to loss of coupling during storage. In accordance with our results it has been shown previously [27], that oligomycin inhibition of the membrane-bound Mg^{2+}-ATPase is quite independent of the coupling state of chromatophore membranes.

High oligomycin-sensitivity both with Mg^{2+} and Ca^{2+} of the solubilized complex indicates the intact functional association of subunit proteins necessary for oligomycin-sensitivity conferring and inhibitor binding. The binding site, or perhaps group of binding sites, for oligomycin and related compounds appears to be located on the F_0 moiety of the *R. rubrum* ATPase [18]. This agrees well with data from the mitochondrial ATPase, where an isolated membraneous subunit as well as the whole F_0 entity has been shown to specifically interact with oligomycin [8].

The molecular weight of the nearly pure ATPase complex has been shown to be above 450,000 [28]. The protein profile of the complex and molecular weights of subunit proteins are in fairly good agreement with those reported for oligomycin or DCCD sensitive ATPases from other sources (2 – 4). Three of the smaller polypeptides (22,000, 20,000, and 11,000) may be components of the hydrophobic part of the enzyme. But further work is required to understand more clearly the role of the subunits present in the purified complex.

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