EFFECT OF AUROVERTIN ON ENERGY TRANSFER REACTIONS IN *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES

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1. Introduction

Aurovertin like oligomycin, was introduced by Lardy et al. [1] as an inhibitor of the synthesis of ATP in mitochondria. However, both antibiotics differ in some of their effects on mitochondrial energy transfer reactions. Aurovertin is a better inhibitor of the reactions leading to ATP synthesis than of the ATP utilizing reactions [1,2]. Moreover, it inhibits the mitochondrial soluble ATPase while oligomycin does not [3]. Therefore, their sites of action are different: aurovertin acts on the coupling factor 1 (F_1) while oligomycin acts on the ATPase complex [3]. When aurovertin binds to mitochondria or to soluble F_1 its fluorescence is greatly enhanced [4]. This property has lead to the use of aurovertin as a conformational probe of the mitochondrial ATPase [4–6].

Oligomycin inhibits energy transfer reactions in *R. rubrum* chromatophores including photophosphorylation, oxidative phosphorylation and partial reactions, while other reactions like photosynthetic pyrophosphate formation and the activity of the soluble ATPase are not affected [7–9].

In this paper we report a study of the effects of aurovertin on the energy transfer reactions of *R. rubrum* chromatophores and on the membrane-bound and soluble ATPases. It is concluded that aurovertin is better than oligomycin as inhibitor of these reactions and that its site of action may be the *R. rubrum* coupling factor.

2. Experimental

Rhodospirillum rubrum cells (the blue-green mutant strain BG-1 kindly given to us by A. García, Universidad de Buenos Aires) were grown anaerobically in the light at 30°C as described [10]. The harvested cells were washed twice with distilled water and suspended in 250 mM sucrose, 20 mM Tris–HCl (pH 8) and 5 mM $MgCl_2$. Chromatophores were prepared by disrupting the cells by ultrasonic irradiation for 2 min at 4°C in a MSE 100W ultrasonic desintegrator followed by centrifugation at 20 000 *g* for 30 min to separate cell debris. Chromatophores were sedimented at 105 000 *g* for 60 min, washed once in the same medium suspended in a small volume and stored in liquid nitrogen. The concentration of bacteriochlorophyll was determined using the extinction coefficient of 140 mM^{-1} cm^{-1} at 873 nm [11].

The basic reaction medium (1 ml) was 30 mM Tris–HCl (pH 8), 5 mM $MgCl_2$ and chromatophores corresponding to 10 μg of bacteriochlorophyll. For photophosphorylation the medium was supplemented with 2 mM ADP and 4 mM Pi containing 10^6 cpm of ^{32}P. In succinate or PMS-mediated photophosphorylation, 30 μM succinate or 300 μM phenazine methosulfate (PMS) plus 1 μM 2-heptyl-4-hydroxy-quinoline-*N*-oxide were present.

The dark or light ATP-Pi exchange reaction was measured in the basic reaction medium plus 4 mM ATP, 7 mM Pi (containing 1×10^7 cpm ^{32}P) and 400 μM PMS (only in the light reaction). The reaction time for photophosphorylation and exchange reactions was 5 min and the temperature 25°C. Light (20 000 lux) was provided by two 150 W tungsten lamps and filtered through 5 cm of water. The ^{32}P incorporated into ATP was determined by an isobutanol-benzene extraction method [12] and counted in a Beckman

LS-233 liquid scintillation counter.

Oxidative phosphorylation was measured in the dark at 25°C for 10 min in a reaction medium (1 ml) containing 30 mM 2 (N-morpholino) ethane sulfonic acid-NaOH (pH 7), 5 mM $MgCl_2$, 0.5 mM ATP, 10 mM Pi (containing 5×10^6 cpm ^{32}P), hexokinase (200 μg), 20 mM glucose, 0.5 mM NADH and chromatophores corresponding to 10 μg of bacteriochlorophyll. The ^{32}P incorporation into organic compounds was determined as above.

The activity of the soluble, Ca-ATPase was determined as described [13] except that the final volume was 0.2 ml and Pi was determined according to Sumner [14].

NAD reduction by succinate and the transhydrogenase reactions were followed spectrophotometrically essentially as described [15,16].

Aurovertin (mol. wt 490) was used in ethanolic solutions with adequate controls for the solvent. CCP was a gift of Dr P. G. Heytler, E. I. Du Pont de Nemours & Co (Wilmington, USA). Oligomycin, ADP and ATP were obtained from Sigma Chemical Co (St. Louis, Missouri, USA). All other reagents were of analytical grade.

3. Results and discussion

Fig.1 shows the effect of increasing concentrations of aurovertin on the endogenous, succinate- and PMS-catalyzed photophosphorylation in *Rhodospirillum rubrum* chromatophores. All the titration curves were similar. The aurovertin concentration for half-maximal inhibition was about 0.1 μM.

Chromatophores from light-grown cells of *R. rubrum* are able to carry out synthesis of ATP in the dark associated with the aerobic oxidation of NADH [17] and catalyze a ATP-Pi exchange reaction which is greatly stimulated by light [18]. As shown in fig.2 all these reactions were inhibited by aurovertin in the same range of concentration as photophosphorylation. This similarity is not surprising since the same coupling factor is probably involved in the photosynthetic and oxidative (dark) phosphorylation [19].

At variance with the nearly complete inhibition by aurovertin of the phosphorylation and ATP-Pi exchange reactions, the antibiotic was considerably less effective as an inhibitor of another partial reaction, namely the ATPase. The dark Mg-ATPase and the CCP-stimulated

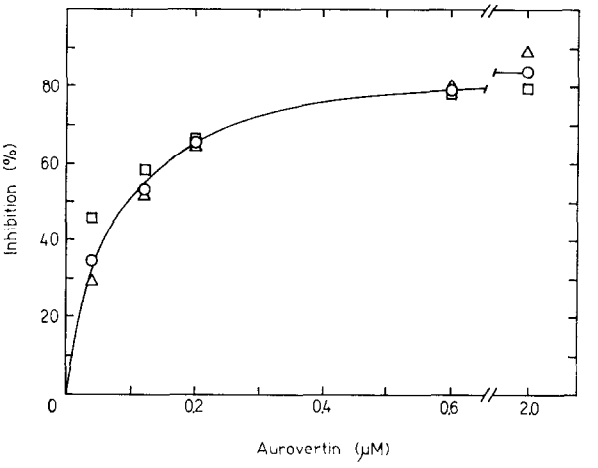

Fig.1. Effect of aurovertin on photophosphorylation in *R. rubrum* chromatophores. Experimental conditions were as described in the text. Control values were 58, 108, 313 μmol/mg bacteriochlorophyll/h for endogenous (□–□) succinate (○–○) and PMS (△–△) photophosphorylation systems.

ATPase [18] activity were partially inhibited by aurovertin (not more than 45% inhibition with 4–10 μM aurovertin). The ATPase of *R. capsulata* chromato-

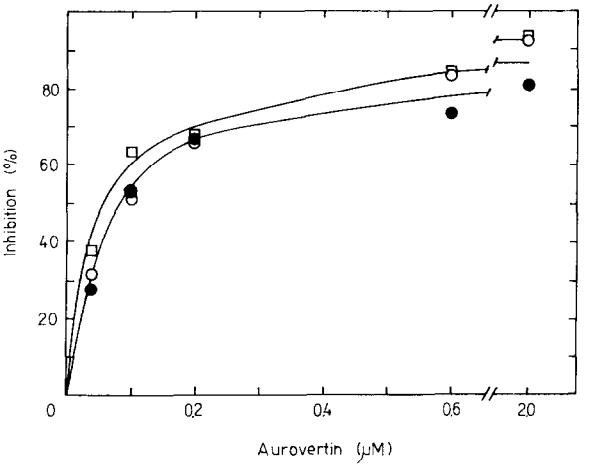

Fig.2. Effect of aurovertin on oxidative phosphorylation and on the dark and light ATP-Pi exchange reactions in *R. rubrum* chromatophores. Experimental conditions were as described in the text. Control values were 6.6, 5.6 and 32.0 μmol/mg bacteriochlorophyll/h for oxidative phosphorylation (□–□) dark (●–●) and light (○–○) ATP-Pi exchange reactions.

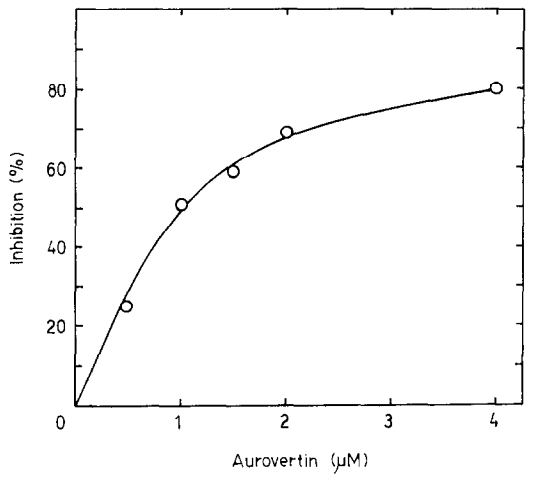

Fig.3. Effect of aurovertin on the soluble Ca-ATPase of *R. rubrum*. Experimental conditions were as described in the text. The control value was 5.90 μmol/mg bacteriochlorophyll/h.

phores has been reported [20] to be partially inhibited by aurovertin only in the presence of Pi.

Johansson et al. [13] have purified from *R. rubrum* chromatophores, a Ca-dependent soluble ATPase which, when added to decoupled chromatophores, restored photophosphorylation and Mg-dependent ATPase activities. This soluble ATPase, at variance with the membrane-bound ATPase, was more sensitive to aurovertin. Fig.3 shows the 4 μM aurovertin inhibited 80% of the activity of this soluble ATPase. The I_{50} was about 1 μM, i.e., ten times higher than for oxidative phosphorylation or photophosphorylation (figs 1 and 2).

Like the membrane-bound (dark) ATPase other ATP-dependent reactions were only partially inhibited by aurovertin. The ATP-driven transhydrogenase reaction was decreased from 34 to 23 μmol NADPH/mg of bacteriochlorophyll/h by 4 μM aurovertin while the light- and PPi-driven reactions were not affected. Similarly, the reduction of NAD by succinate driven by ATP was partially inhibited while the light and PPi dependent reactions were not affected.

Dio-9 and phloridzin have been reported [21] to inhibit to the same extent, like aurovertin (fig.1), three systems of photophosphorylation by chromatophores. However, the concentration of Dio-9 and phloridzin required were quite higher. Moreover, Dio-9 behaved like an uncoupler rather than an energy transfer inhibitor since it inhibited photoreduction of NAD by succinate.

Oligomycin and aurovertin are equally effective as inhibitors of mitochondrial oxidative phosphorylation [1,2]. However, synthesis of ATP in *R. rubrum* chromatophores is more sensitive to aurovertin than to oligomycin. Like earlier shown for the wild strain [7] photophosphorylation in the BG-1 mutant chromatophores was nearly completely inhibited by 8 μM oligomycin (the I_{50} was 1 μM, experiments not shown) i.e., about ten times higher concentrations than those required of aurovertin (fig.1).

Aurovertin does not react with the ATPase from *Escherichia coli* or with choloplast CF_1 [22] but unlike oligomycin [9] inhibits the *R. rubrum* soluble ATPase. Therefore, the site of action of aurovertin in chromatophores, as in mitochondria seems to be the coupling factor. This, together with its high potency as an inhibitor suggests that aurovertin should be a useful tool in studying energy conservation in photosynthetic bacteria.

After this work was completed our attention was called to a report by Gromet-Elhanan [23] who has also found that aurovertin acts as an energy transfer inhibitor in *R. rubrum* chromatophores. In addition, she found that the fluorescence of aurovertin did not increase in the presence of chromatophores or soluble coupling factor.

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