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# ENERGY TRANSDUCTION IN PHOTOSYNTHETIC BACTERIA

## VIII. ACTIVATION OF THE ENERGY-TRANSDUCING ATPase BY INORGANIC PHOSPHATE

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SUMMARY

ATPase activity and ATP-induced energization of photosynthetic membranes from *Rhodopseudomonas capsulata* are stimulated by phosphate; the maximum stimulatory effect occurs at a concentration between 1 and 2 mM.

The sensitivity of the ATPase to oligomycin increases in the presence of phosphate since all the $P_i$-stimulated activity is inhibited by this antibiotic. Aurovertin, which has no effect on ATPase in the absence of phosphate, inhibits completely the activity elicited by this anion.

The addition of $P_i$ induces a substantial increase in the $V$ of ATPase activity without changing the affinity of the enzyme for ATP or ADP.

Arsenate, at the same concentrations, produces effects very similar to those of phosphate. The stimulation by arsenate of the transfer of energy from ATP to the membrane suggests a non-hydrolytic role of this anion as a modifier of the ATPase activity.

## INTRODUCTION

The ATP-hydrolyzing activity of the coupling enzymes in two of the most extensively studied phosphorylating systems, mammalian mitochondria and spinach chloroplasts, appears to be modulated by the presence of inorganic phosphate ($P_i$) in the medium. In rat liver mitochondria, the addition of inorganic phosphate causes a marked acceleration of the maximal velocity of ATPase activity and simultaneously induces a time-dependent change in the $K_i$ for ADP, without altering the affinity of the enzyme for ATP [1].

The $Mg^{2+}$-dependent, light-triggered ATPase of spinach chloroplasts is also affected by both ADP and $P_i$, with ADP causing an acceleration of the rate of decay of the light-triggered state and $P_i$ stabilizing the same state [2].

In photosynthetic membranes from the facultative photosynthetic bacterium, *Rhodopseudomonas capsulata*, photophosphorylation is catalyzed by an ATPase

which resembles spinach chloroplast coupling factor in that it is reversibly activated by light [3]. This effect appears to be mediated by photosynthetic electron transport and to be related to an energized state of the membrane [4].

The observations described here show that this ATPase activity can be affected by inorganic phosphate or arsenate. These anions significantly increase the $V$ of the ATPase without changing the affinity of the enzyme for ATP or ADP. These results represent additional evidence of the analogy between the bacterial ATPase and the above mentioned coupling enzymes, and support further the concept of a substantial unity in the structure and function of energy-transducing ATPases in eukariotic and prokariotic systems.

## MATERIALS AND METHODS

Chromatophores were prepared from photoheterotrophically grown cells of *Rhodopseudomonas capsulata*, strain St. Louis (American Type culture collection n. 23782) by conventional procedures [5]. The standard assay medium for measuring ATPase activity contained in a final volume of 1 ml: Tris–acetate, pH 8.0, 100 $\mu$moles, $MgCl_2$, 5 $\mu$moles, and membrane fragments corresponding to 30–50 $\mu$g of bacteriochlorophyll. All components were incubated in the dark for 5 min at 30 °C; the reaction was started by addition of 0.1 ml of 0.02 M [$\gamma$-$^{32}$P] ATP and was terminated after 3–5 min by addition of 0.2 ml of 25 % trichloroacetic acid. In all of the experiments, ATPase activity was measured in the dark. The amount of $^{32}P_i$ liberated was measured as described by Horio et al. [6].

The quenching of the fluorescence of 9-amino acridine was measured as previously reported [7].

## RESULTS

The effect of addition of 2 mM $P_i$ on the ATPase of photosynthetic membranes from *Rps. capsulata* is shown in Table I. Inorganic phosphate increases ATPase rate about two-fold. The phosphate-stimulated activity is entirely sensitive to oligo-

TABLE I

EFFECT OF ENERGY TRANSFER INHIBITORS AND FCCP ON PHOSPHATE ACTIVATION OF ATPase

Activities are expressed as $\mu$moles ATP hydrolyzed · $h^{-1}$ · $mg^{-1}$ bacteriochlorophyll.

| Additions | ATPase | | | |
|---|---|---|---|---|
| | $-P_i$ | | +2 mM $P_i$ | |
| | Activity | % control | Activity | % control |
| None | 18.0 | — | 35.5 | — |
| 23 $\mu$M oligomycin | 8.0 | 44.4 | 9.1 | 25.6 |
| 0.1 $\mu$M FCCP | 27.8 | 155 | 39.0 | 110 |
| None | 29 | — | 51.1 | — |
| 2.5 $\mu$M aurovertin | 28.5 | 94.5 | 38.5 | 67.5 |

mycin. Aurovertin, another well known energy-transfer inhibitor which acts directly on coupling factor 1 of mitochondria [8], inhibits bacterial photophosphorylation (data not shown). However, whereas oligomycin inhibits the ATPase either in the presence or the absence of $P_i$, aurovertin exerts its inhibitory effect only on the $P_i$-stimulated activity and has no effect on the ATPase in the absence of $P_i$. These results agree with observations made by Mitchell and Moyle [1] in rat liver mitochondria, where phopsphate-activated ATPase is depressed to the phosphate-free level by aurovertin.

An activation of the ATPase by $P_i$ occurs also in the presence of $10^{-7}$ M carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), a concentration which stimulates maximally the activity of the enzyme (Table I), but the stimulation by the uncoupler is reduced in the presence of phosphate. These responses to energy-transfer inhibitors and to uncouplers are qualitatively similar to those obesrved for the light-activated ATPase of bacterial chromatophores [3]. Because $P_i$ stabilizes the light-triggered activated state in spinach chloroplasts [2], we examined the effect of $P_i$ on the bacterial ATPase during a light-to-dark transition. No stabilization of the light-activated state could be detected.

In order to define better the parameters influenced by $P_i$, we have examined the kinetic behaviour of the enzyme as a function of ATP and ADP concentrations. $P_i$ does not change the affinity for ATP ($K_m = 0.11$ mM) but only increases the $V$ of the enzyme from 21.6–34.0 $\mu$moles $\cdot$ $h^{-1}$ $\cdot$ $mg^{-1}$ bacteriochlorophyll. Neither does $P_i$ cause any significant effect on the competitive inhibition of the ATPase by ADP

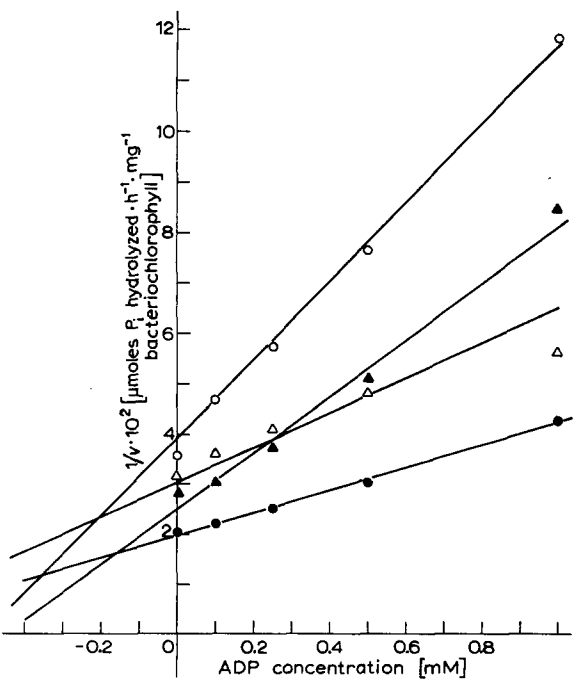


Fig. 1. Linear competitive inhibition of ADP with ATP in the ATPase reaction measured in the absence or in the presence of phosphate. ○–○, 0.2 mM ATP; △–△, 0.5 mM ATP; ▲–▲, 0.2 mM ATP and 1 mM $P_i$; ●–●, 0.5 mM ATP and 1 mM $P_i$.

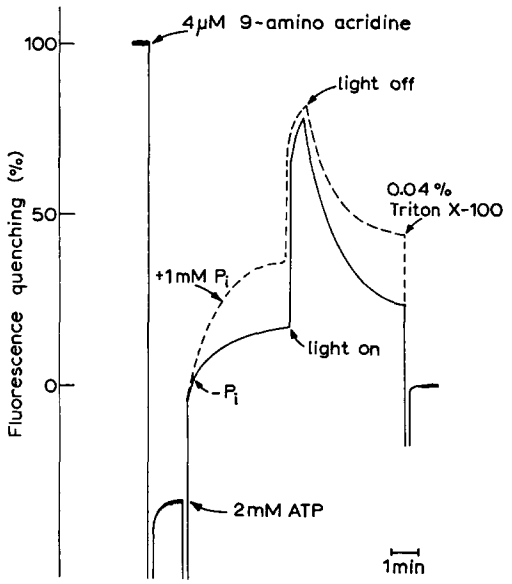


Fig. 2. Effect of 1 mM phosphate on the ATP-driven quenching of the fluorescence of 9-amino acridine by *Rps. capsulata* chromatophores. The assay contained in a final volume of 2.5 ml: glycyl-glycine buffer, pH 7.8, 80 μmoles; $MgCl_2$ 12.5 μmoles; KCl, 250 μmoles: valinomycin, 4 μg; 9-amino acridine, 10 nmoles; ATP, 5 μmoles; chromatophores corresponding to 42 μg bacteriochlorophyll. The degree of quenching was evaluated taking as 100 per cent fluorescence the signal obtained in the presence of ATP and 0.04 % Triton X-100, since a direct effect of ATP on the fluorescence of the amine was observed.

(Fig. 1). The apparent $K_i$ for ADP measured in phosphate-free assays or in the presence of 1 mM orthophosphate at pH 8 were 0.15 and 0.16, respectively, in good agreement with previously reported data [3].

The stimulatory effect of $P_i$ on the rate of ATP hydrolysis can also be demonstrated by measuring the rate and the steady-state level of energization of the membrane, that is induced by addition of ATP in the dark. The energization has been evaluated by examining the ATP-induced quenching of fluorescence of 9-amino acridine [7], a phenomenon related to proton translocation.

Fig. 2 shows that the addition of 2 mM ATP, after a decrease of the fluorescence due to a non-enzymatic interaction between the amine and ATP itself, causes a slow decrease in fluorescence which reaches a steady state after about 3 min. The steady-state level of the quenching was evaluated better after a short burst of actinic light, to bring the membranes to a level of energization greater than that which ATP alone could maintain. The addition of 1 mM $P_i$ stimulates significantly, both the initial rate and the steady-state level of proton translocation. These experiments were performed in the presence of 100 mM KCl and valinomycin in order to minimize any influence of penetrating ions on the rate of proton translocation. Under these conditions, additions of other permeant anions do not induce any stimulatory effect on the quenching of fluorescence of 9-amino acridine, that is induced by ATP in the dark.

Fig. 3 shows the quantitative correspondence between the stimulation of the

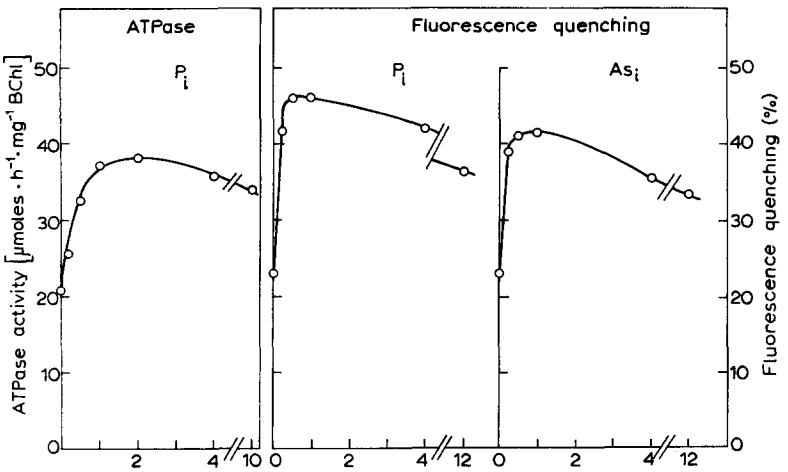


Fig. 3. Stimulation of ATPase activity and of the ATP-driven quenching of 9-amino acridine fluorescence as a function of phosphate or arsenate concentration. Conditions for the quenching assays as in Fig. 3. BChl, bacteriochlorophyll.

ATPase and of the ATP-driven proton translocation, as a function of phosphate concentration. The same figure also shows that arsenate produces effects very similar to those of phosphate. The maximal stimulation is reached between 1 and 2 mM in all cases, at a concentration range comparable with the $K_m$ for phoshate and the $K_i$ of arsenate for the competitive inhibition of photophosphorylation [9] and comparable with that, at which maximal stimulation of light-induced proton uptake by $As_i$ is observed [9]. In agreement with the results shown for the quenching of 9-amino acridine fluorescence, arsenate also stimulates the ATPase activity at comparable concentrations (Table II). When the ATPase was measured in the dark, arsenate increased the rate as much as 226 per cent. This stimulation also could be observed under conditions of light activation, although generally to a lower extent. The effect of phosphate on light-ATPase cannot be studied easily, due to the interference of a light-driven ATP–$^{32}P_i$ exchange reaction, which is very active in these chromatophore preparations [5].

Stimulatory effects on the ATPase by organic anions (especially dicarboxylic acids) were also observed. These effects were clearly different from those of $P_i$ or arsenate, in that no stimulation could be observed at pH 8, the pH optimum being around 7.3. In addition the maximal stimulation required very high concentrations of the anions (e.g. 100 mM for malonate).

TABLE II

EFFECT OF ARSENATE ON LIGHT OR DARK ATPase ACTIVITY

Activities are expressed as μmoles ATP hydrolyzed · $h^{-1}$ · $mg^{-1}$ bacteriochlorophyll.

| Additions | Light ATPase | | Dark ATPase | |
|---|---|---|---|---|
| | Activity | % control | Activity | % control |
| None | 54.3 | — | 20.1 | |
| Arsenate (2 mM) | 81.5 | 151 | 45.5 | 226 |

## DISCUSSION

The results described above support the concept that $P_i$ is an important modifier of the ATPase in membranes of photosynthetic bacteria. The specificity of the effect and the low concentration at which the stimulation of activity occurs, suggest that this phenomenon may be relevant to the mechanism of the regulation of photophosphorylation. The response of the bacterial enzyme to $P_i$ is similar to that observed in rat liver mitochondria [1]. It is also consistent with the properties of the spinach chloroplast enzyme (cf. ref. 2, Table IV). The light-triggered state of the chloroplast ATPase is stabilized by $P_i$, but we have not been able to demonstrate that this holds also for the bacterial enzyme, because the light-triggered state is not stable in the dark.

Arsenate can substitute for phosphate in the stimulation of the ATPase and of the energization driven by ATP. A stimulation of ATPase activity by arsenate had been observed previously in *Rhodospirillum rubrum* chromatophores by Horio et al. [10]. They interpret this effect as due to an uncoupling, involving arsenolytic breakdown of a hypothetical intermediate. Arsenate was also shown to inhibit photophosphorylation in *Rps. capsulata* chromatophores as a competitive inhibitor against phosphate [9]. In the light of our results, a simple hydrolytic role of arsenate is not easy to defend, since concentrations of arsenate as high as 12 mM stimulated ATP-driven proton translocation, rather than inhibiting it. Our results seem to indicate instead that the stimulation of ATPase by arsenate (and phosphate) involves an accelerated transfer of energy from ATP to the membrane. This conclusion agrees with those of Mitchell and Moyle regarding rat liver mitochondria [1]. A non-hydrolytic effect of arsenate in energy-transducing membranes also has been suggested by Huang and Mitchell [11] on the basis of the observation that arsenate can stimulate the ATP-driven reduction of NAD by succinate in beef heart submitochondrial particles. On the same line of indirect evidence the stimulatory effect of arsenate (together with ADP) on the proton uptake induced by light in bacterial chromatophores [9] and higher plant chloroplasts [12] must also be considered.

In previous papers [3, 4] we have investigated the nature of the stimulation by light of the ATPase in *Rps. capsulata* chromatophores. The possibility arises that activations by phosphate and by light may be two aspects of the same phenomenon, an interpretation which has been offered for the case of spinach chloroplasts [2]. Indeed both activations present several interesting features in common: both phosphate- and light-induced activities are sensitive to oligomycin and aurovertin; both involve an approximately two-fold increase in $V$, the affinity for ATP being unchanged; both activations are relatively less evident under uncoupling conditions (e.g. 0.1 $\mu$M FCCP). Other observations, however, do not agree with this conclusion. The activation by light, which obviously requires the interaction of the coupling factor with the membrane [13], reduces, but does not mask completely, the activation by arsenate. The stimulation by phosphate can be measured also for the low ATPase activity of the solubilized coupling factor (data not shown). More difficult to reconcile is the finding that light is a requirement for eliciting the sensitivity of the enzyme towards *N*-ethylmaleimide, while inorganic phosphate or arsenate protect the enzyme from this type of inhibition [4]. The protection by phosphate, however, could be interpreted either as an effect of the binding of a substrate to the active site, or as the consequence of a

conformational change of the protein. ADP exerts a similar protection against *N*-ethylmaleimide, but inhibits the activation by light [3].

The roles of phosphate, ADP and light in modulating the activity of the ATPase will require further study for clarification.

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