Short Communication

ΔpH- and $\Delta\Psi$-Induced ATP and PPi Synthesis in *Rhodospirillum rubrum* Chromatophores*

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Acid-base transitions have been used since 1965[1] to confirm the concepts of the chemiosmotic theory. Chloroplasts[1,2] were equilibrated at low pH and then transferred to a more alkaline solution. The artificial pH-gradient created in this way was shown to drive the formation of ATP by the ATPase. Over the years, ATP-synthesis induced by imposing artificial ion gradients has been demonstrated for mitochondria (Ref. 3 as interpreted by Glynn,[4] and Refs. 5 and 6), submitochondrial particles[7] and bacterial chromatophores.[8]

The membrane-bound pyrophosphatase in chromatophores from *Rhodospirillum rubrum* serves as a catalyst for the formation or hydrolysis of inorganic pyrophosphate coupled to the proton motive force. The common feature of the ATPase and the PPase, viz. utilization of the proton motive force for synthesis of their respective energy-rich products, would make it possible to compare the actions of the two enzymes when the chromatophores are subjected to artificial electric gradients ($\Delta\Psi$) and pH-gradients (ΔpH).

Experimental

Preparation of chromatophores from light-grown *R. rubrum* was carried out as described previously,[9] with the exceptions that a Ribi press was used to rupture the bacterial cells and that 0.2 M glycylglycine (pH 7.4) was used to wash and suspend the chromatophores. For continuous assay of ATP synthesis, the luciferin/luciferase-based ATP-monitoring kit (*LKB-Wallac, Turku, Finland*) was used. PPi formation was monitored by a new continuous method, introduced by Nyrén *et al.*,[10] which also involves luciferin and luciferase. The chemicals used in this assay were from the same sources as given in Ref. 10. The assay medium was placed in a LKB-Wallac 1250 luminometer at room temperature.

Chromatophores were equilibrated at pH 5.3 for 2 min in a medium containing 0.2 M glycylglycine and 1 mM Na-succinate. When ATP synthesis was monitored, chromatophores corresponding to 1–2 μm bacteriochlorophyll (Bchl) were transferred to a tube within the luminometer; the 0.5 ml of assay medium contained 0.2 M glycylglycine, 10 mM K-Pi, 50 μM ADP, 1 μM P^1,P^5-di(adenosine-5′)pentaphosphate (DAPP, inhibitor of the competing myokinase reaction), 50 μl of ATP-monitoring reagent, 10 μM valinomycin and KCl, the amount of which depended on the K^+-gradient desired. The pH of this medium was 8.3 unless otherwise stated. When PPi was assayed, the 0.5 ml of assay medium contained 0.2 M glycylglycine, 10 mM Pi, 1 μM DAPP, 5 μg of oligomycin, 1 mM 1,4-dithioerythritol, 0.3 U of ATP-sulfurylase, 5 μM adenosine-5′-phosphosulfate, 1–2 μM Bchl, 10 mM $MgAc_2$, 0.1 % BSA, 0.05 mg of D-luciferin and 4 μg of L-luciferin. The Pi used was Na-Pi when no electrical gradient was desired and K-Pi elsewhere. When a K^+ gradient was used, 10 μM valinomycin and varying concentrations of KCl were also

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included in the medium. The pH of the medium here was also 8.3 unless otherwise stated.

Results and discussion

Fig. 1 shows the amount of ATP formed as a function of the potassium ion concentration. Two different ΔpH were used, 0 and 2.7. Measurement of ATP synthesis at ΔpH 2.7 without any K^+ diffusion potential present was not possible since this gave rise to strange behaviour of the system. However, extrapolation of the curve to 0 mM K^+ shows that a pH-gradient without an accompanying electric gradient is not sufficient to induce ATP synthesis. This is consistent with previous findings.[8] It can also be seen that a potassium ion concentration of 50 mM was incapable of inducing ATP synthesis when there was no pH-gradient present. This could be interpreted as showing a requirement of a minimum value of the proton motive force before ATP-synthesis can be accomplished. Such a threshold requirement has also been proposed for chloroplast ATPases.[2,11-14]

In contrast to Fig. 1, Fig. 2 shows that PPi synthesis can be induced by a pH-gradient alone and that there is no threshold for PPi synthesis at low electric gradients.

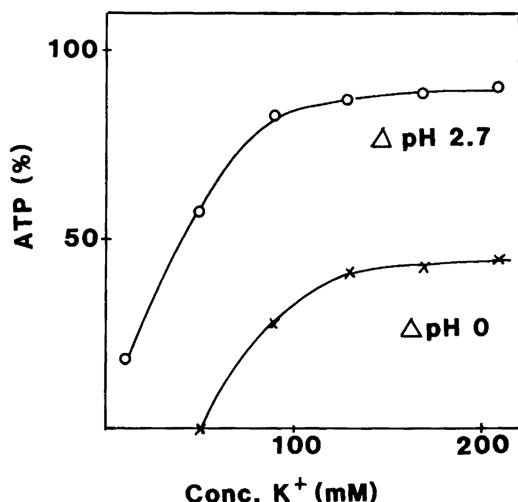

Fig. 1. Amount of ATP formed as a function of the K^+ concentration in the assay buffer. The points are mean values and the largest amount of ATP formed is taken as 100 %.

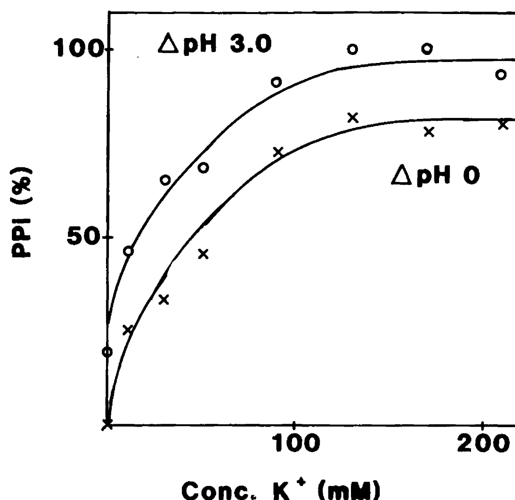

Fig. 2. Amount of PPi formed as a function of the K^+ concentration in the assay buffer. The points are mean values and the largest amount of PPi formed is taken as 100 %.

The amount of PPi and ATP synthesized, as in Figs. 1 and 2, varied among different preparations, but typical values for the maximal amounts synthesized were 20 nmol ATP (mg Bchl)$^{-1}$ and 200 nmol PPi (mg Bchl)$^{-1}$, a ten-fold greater production of PPi than of ATP. Even at 10 times higher ADP concentrations there was no increase in ATP formation. ATP synthesis could be seen to occur for a period of 20–30 sec after injection of the low pH chromatophores into the assay medium, while the PPi synthesis was continued up to 10 min. These results could reflect: (1) the differences in $\Delta G^{o'}$ for the two reactions [4.0 kcal mol^{-1} for formation of PPi at 1 mM of free Mg^{2+} (see Ref. 15) and 7.3 kcal mol^{-1} for ATP synthesis[16]], (2) differences in the amounts of the two relevant enzymes in the chromatophore membranes, (3) different numbers of protons that need to be translocated by the two enzymes for the formation of one high-energy molecule, and (4) a necessary activation of the ATPase by a large membrane potential before it can catalyze ATP formation.

Under the conditions applying for ATP synthesis, it is possible that the artificially created gradients could be partially dissipated through the PPase. Abolishment of PPi synthesis can be accomplished by adding fluoride ions. Table 1 re-

Table 1. ATP and PPi formation when inhibitors of the ATPase (oligomycin) and the PPase (fluoride ions) were present or absent. The oligomycin concentration was 10 μg ml^{-1}. F^- was present at 10 mM. The values corresponding to the conditions used in Figs. 1 and 2 are taken as 100%. ΔpH was 3.0 and the potassium ion concentration in the assay medium was 170 mM. The maximal amounts of ATP and PPi formed according to Figs. 1 and 2 were taken as 100%.

Addition	PPi	ATP
Control	93%	100%
+Oligomycin	100%	0
+NaF	0	113%

veals a small enhancement in the amount of ATP synthesized when F^- is included. The measurement of PPi formation was performed with oligomycin present to inhibit the consumption of protons by the ATPase and to prevent leakage through uncoupled ATPases. When oligomycin was not added, the formation of PPi was somewhat diminished (see Table 1).

We have shown that the synthesis of large amounts of inorganic pyrophosphate (compared to the amounts of ATP formed) can be induced by artificially created pH-gradients and electric gradients, applied either separately or simultaneously. In contrast to the ATP synthesis induced by these artificial gradients, no threshold membrane potential seems to be needed for PPi synthesis. Our suggestion is that pyrophosphate formation requires a smaller proton motive force than ATP synthesis, reflecting the smaller $\Delta G^{o\prime}$ for the reaction. The enzyme PPase requires no activation step, as has been implied by us previously for PPi hydrolysis,[17] and we would also like to consider the possibility that the PPase might need a smaller number of protons to synthesize PPi than is required by the ATPase to synthesize ATP.

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