PROTON-TRANSLOCATING PYROPHOSPHATASE
OF *RHODOSPIRILLUM RUBRUM*

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1. Introduction

The membrane of chromatophores of *Rhodospirillum rubrum* contains an oligomycin-insensitive reversible pyrophosphatase system [1] that is involved in energy transduction by a pathway separate from that of the oligomycin-sensitive ATPase system [2–6]. Pyrophosphate hydrolysis by chromatophores induces a change of carotenoid absorbance [7], a change in the fluorescence of added 8-anilinonaphthalene-1-sulphonic acid [8] and an uptake of phenyl dicarbaundecaborane anion [9]. Therefore it has been inferred that pyrophosphate hydrolysis by the pyrophosphatase system is coupled to (electrogenic) ion translocation across the chromatophore membrane; but the species of ion involved has not yet been identified.

In this paper we describe measurements of changes of the pH and pK of the outer medium (pH_O and pK_O) during the hydrolysis of pulses of inorganic pyrophosphate (PP_i) by suspensions of chromatophores from *R. rubrum* under various conditions, and we show that the pyrophosphatase system translocates protons, as suggested earlier [10, 11].

Abbreviations:
  pH: $-\log_{10}$ (chemical activity of H^+);
  pK: $-\log_{10}$ (chemical activity of K^+);
  FCCP: carbonylcyanide *p*-trifluoromethoxyphenylhydrazone;
  PP_i: inorganic pyrophosphate;
  $\rightarrow H^+/PP_i$ quotient: g ion H^+ translocated per mole PP_i hydrolysed.

2. Materials and methods

R. rubrum (NCIB 8255) was grown, harvested and washed, and chromatophores were prepared, by methods similar to those described previously [12]; but the medium used for sonicating the cells and washing the resulting chromatophores was 250 mM sucrose, 3 mM glycylglycine, 9 mM choline chloride, 1 mM KCl, 0.3 mM $MgCl_2$ at pH 7.1.

The pH_O and pK_O measurements during PP_i hydrolysis by the chromatophores were done at 25° in an anaerobic reaction vessel of 2.6 ml capacity, employing our usual type of instrumentation and methods [12].

3. Results and discussion

Fig. 1 shows typical strip-chart recordings of proton pulses following the injection of 200 nmoles of PP_i into anaerobic chromatophore suspensions completely shielded from light. Upper trace A is the pulse obtained when the main electrolyte of the reaction medium was 10 mM KCl and valinomycin was present to collapse the membrane potential. Lower trace A is the corresponding control pulse obtained when the proton conductor FCCP was present (to collapse the electrochemical potential difference of H^+ across the membrane) in place of valinomycin. The initial pH_O change in upper and lower traces A was the same, and it is attributable to the displacement of H^+ from the injected pyrophosphate by the Mg^{2+} present in the medium*.

* This initial pH_O displacement was unavoidable because $MgCl_2$ could not be added to the injected PP_i solution owing to the low solubility of magnesium pyrophosphate.
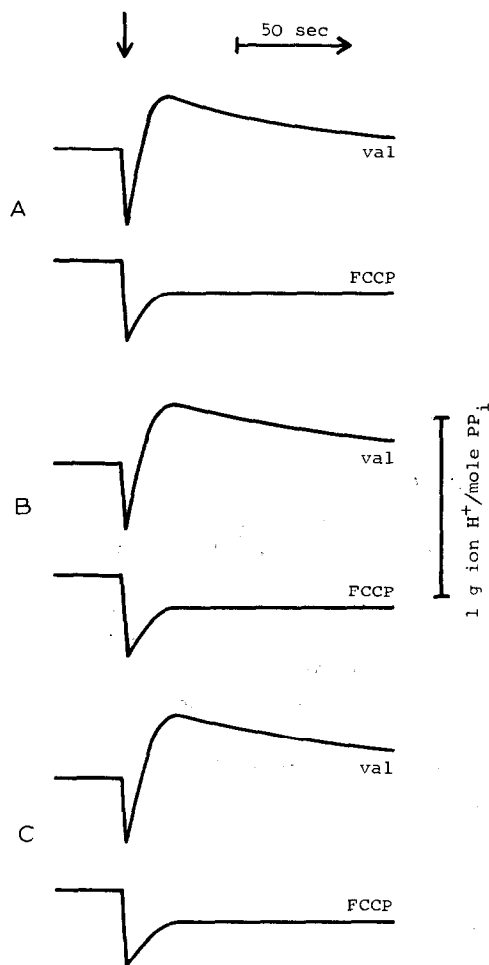
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Fig. 1. Time course of changes of pH_O in suspensions of chromatophores of *R. rubrum* on injection of PP_i and during its subsequent hydrolysis. Chromatophores (4.8 mg protein/ml) were completely shielded from light and suspended in an anaerobic medium, at $25°$ and at pH_O 7.0 to 7.1, containing 250 mM sucrose, 3.3 mM glycylglycine, 0.2 mM $MgCl_2$ and (A) 10 mM KCl, or (B) 9 mM NaCl + 1 mM KCl, or (C) 9 mM choline chloride + 1 mM KCl. Carbonic anhydrase (final conc. 77 μg/ml) and oligomycin (2.9 mg/g chromatophore protein) were routinely added to the suspensions; and valinomycin (val) (200 μg/g chromatophore protein) or FCCP (final conc. 1 μM) were added as indicated in the figure. At the arrow, 200 nmoles of PP_i were injected (as an anaerobic 5 mM solution adjusted to pH 7.05). The strip-chart recordings of changes of pH_O have been brought to the same scale and corrected for baseline drift corresponding to not more than 0.004 pH unit per min. A decrease of H^+ activity in the outer medium is shown as an upward deflection.

The subsequent pH_O change in lower trace A represents the net change of acidity on hydrolysis of the magnesium pyrophosphate, while that in upper trace A also includes the pH_O change attributable to proton translocation, an upward deflection showing inward proton translocation. Thus, the extent of proton translocation can be obtained from the difference between the upper and lower traces. Traces B and C of fig. 1 were obtained in experiments corresponding to those of traces A, but using 9 mM NaCl and 9 mM choline chloride, respectively, as the main electrolyte of the medium, and including 1 mM KCl to permit collapse of the membrane potential when valinomycin was present. All the traces of fig. 1 are scaled to read as g ion H^+/mole PP_i in the pulse; and it will be noted that the apparent $\rightarrow H^+/PP_i$ quotient was about 0.5, independently of the main cation present. The time for half decay of the proton displacement across the membrane was of the order of 100 sec. Proton pulses generated by flashes of light gave the same time of half decay as the proton pulses generated by PP_i hydrolysis.

The concentration of Na^+ in the 10 mM KCl (traces A) and 9 mM choline chloride + 1 mM KCl (traces C) media containing chromatophores was found by flame photometry to be 43 μM and 50 μM, respectively. The lack of significant effect of Na^+ concentration on the apparent $\rightarrow H^+/PP_i$ quotient shows that the observed proton translocation could not have been due to co-operation between Na^+ translocation by the pyrophosphatase and the action of a putative Na^+/H^+ antiporter [11].

As shown in fig. 2, using 10 mM choline chloride as main electrolyte in the reaction medium and at a K^+ concentration corresponding to about $pK_O = 3.3$, when no valinomycin or FCCP were present (traces A), the injection of 200 nmoles of PP_i caused little or no significant H^+ or K^+ translocation. Likewise, in presence of FCCP, traces B show that there was no significant K^+ translocation during the pH_O change corresponding to net change of acidity during PP_i hydrolysis. However, when the membrane was made permeable to K^+ by the presence of valinomycin, traces C show that the inward translocation of protons was accompanied by an outward translocation of K^+. The extent of the H^+ translocation, obtained by subtracting the net pH_O changes given by upper trace B from those of upper trace C is shown in broken
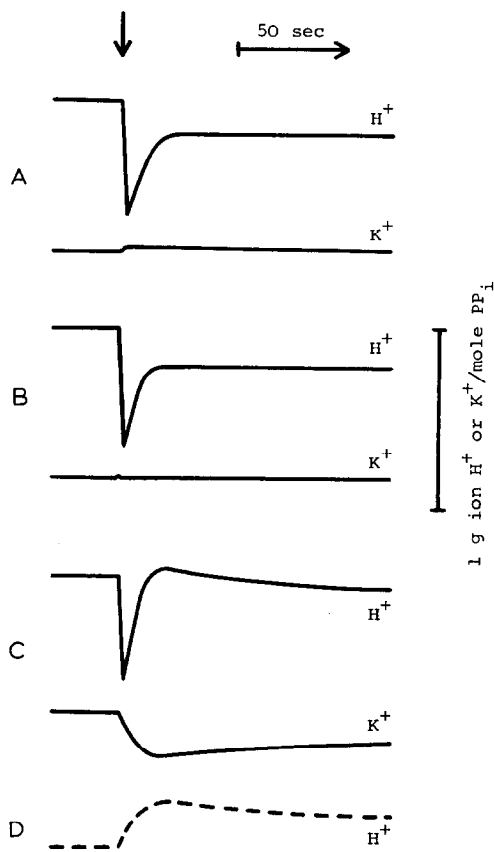
Fig. 2. Traces A, B and C: Time-course of changes of pH_O and pK_O in suspensions of chromatophores of *R. rubrum* on injection of PP_i and during its subsequent hydrolysis. Chromatophores (3.8 mg protein/ml) were completely shielded from light and suspended in an anaerobic medium containing 250 mM sucrose, 3.3 mM glycylglycine, 0.5 mM $MgCl_2$, 10 mM choline chloride, and KCl to give pK_O = 3.22 to 3.28, at $25°$ and at pH_O 7.0 to 7.1. Carbonic anhydrase (77 μg/ml) and oligomycin (3.7 mg/g chromatophore protein) were routinely added to the suspensions. In A, there were no other additions; in B, FCCP (final conc. 1 μM) was added; in C, valinomycin (250 μg/g chromatophore protein) was added. At the arrow, 200 nmoles of PP_i (choline salt) were injected as an anaerobic 5 mM solution adjusted to pH 7.05. The strip-chart recordings of changes of pH_O and pK_O have been brought to the same scale, and pK_O recordings have been corrected for the effect of dilution on injection of the PP_i solution. A decrease of H^+ or K^+ activity in the outer medium is shown as an upward deflection. Curve D: Proton translocation accompanying PP_i hydrolysis, obtained from the difference between pH_O trace C and pH_O trace B.

curve D. Comparison of lower trace C with curve D shows that, in presence of valinomycin, the translocation of K^+ outwards was stoichiometrically equivalent to the translocation of H^+ inwards, estimated from the change of pH_O. We infer that a proton-translocating pyrophosphatase system is involved in the pyrophosphatase activity of chromatophores.

Oligomycin was routinely added to the chromatophores in the experiments described by figs. 1 and 2 because previous work [12] showed that the effective proton conductance of the membrane of certain chromatophore preparations could be decreased, and the apparent stoichiometry of proton translocation could consequently be enhanced, by oligomycin. However, omission of oligomycin did not significantly influence the results of experiments such as those of figs. 1 and 2; and this observation was consistent with the fact that oligomycin did not influence the effective proton conductance of the membrane of the chromatophore preparations described here.

The chromatophore preparations probably contain some disorganised membrane material and it is possible that they may also contain a pyrophosphatase enzyme species [13] that does not translocate protons. It follows that the $\rightarrow H^+/PP_i$ quotient of about 0.5 observed in these chromatophore preparations is probably lower than the actual $\rightarrow H^+/PP_i$ quotient characteristic of the proton-translocating pyrophosphatase system of intact chromatophore membranes of *R. rubrum*.

Acknowledgements

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