LIMITED COOPERATIVITY IN THE COUPLING BETWEEN ELECTRON FLOW AND PHOTOSYNTHETIC ATP SYNTHESIS

A comparative study in chromatophores phosphorylating at very different rates

Rita CASADIO, Assunta BACCARINI MELANDRI and Bruno A. MELANDRI
Institute of Botany, University of Bologna, via Irnerio 42, 40126 Bologna, Italy

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1. Introduction

The kinetics of phosphorylation by chromatophores from a photosynthetic bacterium, *Rhodopseudomonas capsulata,* was investigated [1,2] and correlated with the value of the protonmotive force established in the steady state under continous illumination in a variety of experimental conditions. In these studies the value of Δp was monitored by spectroscopic methods (electrochromic band shift of carotenoids for the evaluation of the membrane potential ($\Delta\psi$) and light-induced quenching of the fluorescence of 9-aminoacridine for an estimate of transmembrane ΔpH) and related to the rates of photophosphorylation measured concurrently.

An unexpected conclusion of this work was that the rate of photophosphorylation was not unequivocally related to the value of Δp (nor to that of $\Delta\psi$ or ΔpH), as expected if a chemiosmotic mechanism of coupling [3] between the redox reactions of photosynthetic electron flow and ATP synthesis operates. In fact, a different relation linked the rate of photophosphorylation (v_p) to Δp in progressively uncoupled vesicles, in which both v_p and Δp were decreased in parallel, as compared to that in electron transport-limited chromatophores, in which a greatly diminished v_p was not accompained by a comparable decrease of Δp.

A possible interpretation of these results, in line with the chemiosmotic coupling hypothesis, as cribes these discrepancies to the presence of a mixed population of vesicles, in which only a small portion were actually active in ATP synthesis. In such a system added uncouplers would affect the steady state extent of Δp in all chromatophores, irrespective of their ability to phosphorylate. On the contrary, under conditions of limited energy input, Δp would collapse only in phosphorylating chromatophores, as a result of the utilization of the photosynthetic gradient by ATP synthetase: this effect would not be observed in the value of Δp averaged on the whole population. A similar possibility was also suggested [4] for reconciling the expectations of the chemiosmotic hypothesis with their observations, that the decay of only a small fraction (about 10–20%) of the carotenoid signal, induced by a flash, was accelerated by ADP and phosphate.

The utilization of a different strain of *Rps. capsulata,* cultured under improved growth conditions, has enabled us to obtain chromatophore preparations capable of high rate of photophosphorylation comparable to those evaluated 'in vivo' [5]. Utilizing such highly active preparations we have re-examined the relations between the rate of ATP synthesis and the

Abbreviations: Bchl, bacteriophyll; DCCD, *N,N'*-dicyclohexylcarbodi-imide; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenyl-hydrazone;

$\Delta p = \Delta\bar{\mu}H^+/F = \Delta\psi - 2.3RT/F\ \Delta$pH,

protonmotive force, i.e., transmembrane difference in the electrochemical potential of protons, expressed in electrical units

Address correspondence to: B. A. Melandri

extent of Δp. The results are compared with those obtained in chromatophores from the same bacterial strain, in which the ability for ATP synthesis had been drastically impaired either by partial detachment of the coupling factor or by treatment with the covalent inhibitor, DCCD.

2. Materials and methods

Cells of *Rps. capsulata,* strain Kbl (kindly supplied by Professor J. H. Klemme, University of Bonn) were grown at 30°C in Roux bottles in the medium [6]. Homogenous illumination of the cultures was obtained by four 150 W incandescent lamps positioned on both sides of the culture bottles; the average light intensity on the surface was 150 J . m^{-2} . s^{-1}, measured with a Yellow Springs Instruments radiometer (model 65 A). Cells were harvested in the late logarithmic phase of growth, chromatophores were prepared as detailed in [7] and stored at −16°C in a glycerol containing buffer (Na-glycylglycine, pH 7.2, 50 mM; $MgCl_2$, 5 mM and glycerol 60%, v/v).

DCCD was incubated for 1 h, at room temperature, with chromatophores suspended in the storage buffer. The inhibited chromatophores were stored at −16°C; under these conditions no change in the inhibited photophosphorylation activity was observed for a period of several hours.

Coupling factor (F_1) was removed by sonication of chromatophores suspended in 1 mM EDTA as in [7].

Measurements of the quenching of 9-aminoacridine, and of the carotenoid band shift were done as in [8,9]. The values of $\Delta\psi$ and ΔpH were calculated from the experimental data using the criteria specified in [2,9]. The rate of photophosphorylation was measured concurrently with the extent of the electrochemical difference of protons by isotopic methods [2]. The medium for these assays was as in [2], except that KCl was omitted since a significant inhibitory effect on photophosphorylation by salts was often noticed.

The medium for the assay of the fluorescence quenching of 9-aminoacridine and of the carotenoid band shift, induced in the dark by ATP hydrolysis, is described in the text.

3. Results and discussion

The chromatophores utilized in these studies are very active in photophosphorylation and can reach rates as high as 900 μmol h^{-1} . mg $Bchl^{-1}$ in saturating light. These rates are comparable with those observed 'in vitro' in other photophosphorylating systems [10,11] and are close to the rate of ATP synthesis measured 'in vivo' in photosynthetic bacteria [5]. The amount of bacteriochlorophyll per protein in these preparations is quite similar to that of *Rps. capsulata,* strain St Louis. The improved activity appears to be due to a larger amount of ATP synthetase bound to the membrane and/or to a tighter coupling of this enzyme, as suggested by the higher ATPase activity measured in these chromatophores (120 μmol $h.^{-1}$ mg $Bchl^{-1}$ when measured in the dark and 230 in the light) and by the greater sensitivity of the ATPase to oligomycin.

With this preparation it has been possible to study the time course of the formation of both components of the protonic gradient ($\Delta\psi$ and ΔpH), induced in the dark by ATP hydrolysis and their response to the addition of ionophores. A typical experiment of this kind is shown in fig.1: under optimal conditions for ATPase activity, i.e., in the presence of 1 mM phosphate [12], addition of ATP in the dark induces a prompt formation of a protonic gradient, which reaches a stationary level in about 4–5 min and which is composed of a large pH difference (2.6 units) and a relatively small membrane potential (about 30 mV). The overall Δp under these conditions amounts to about 185 mV. Upon addition of ionophorous antibiotics both $\Delta\psi$ and ΔpH show a response consistent with the presence of a highly-active electrogenic proton pump coupled to ATP hydrolysis. Addition of oligomycin completely inhibits the formation of the proton gradient, in line with the previous qualitative observations performed with atebrin as a ΔpH indicator [13]. The ATP-induced carotenoid signal, obtained with these preparations of *Rps. capsulata,* confirms that the electric field induced by ATP hydrolysis in the dark is sensed by the endogenous pigments [14]; this observation strengthens the view that the carotenoid band shift is a valid indicator of membrane potential, whatever the mechanism is for the generation of this potential.

The relation between the rate of photophos-

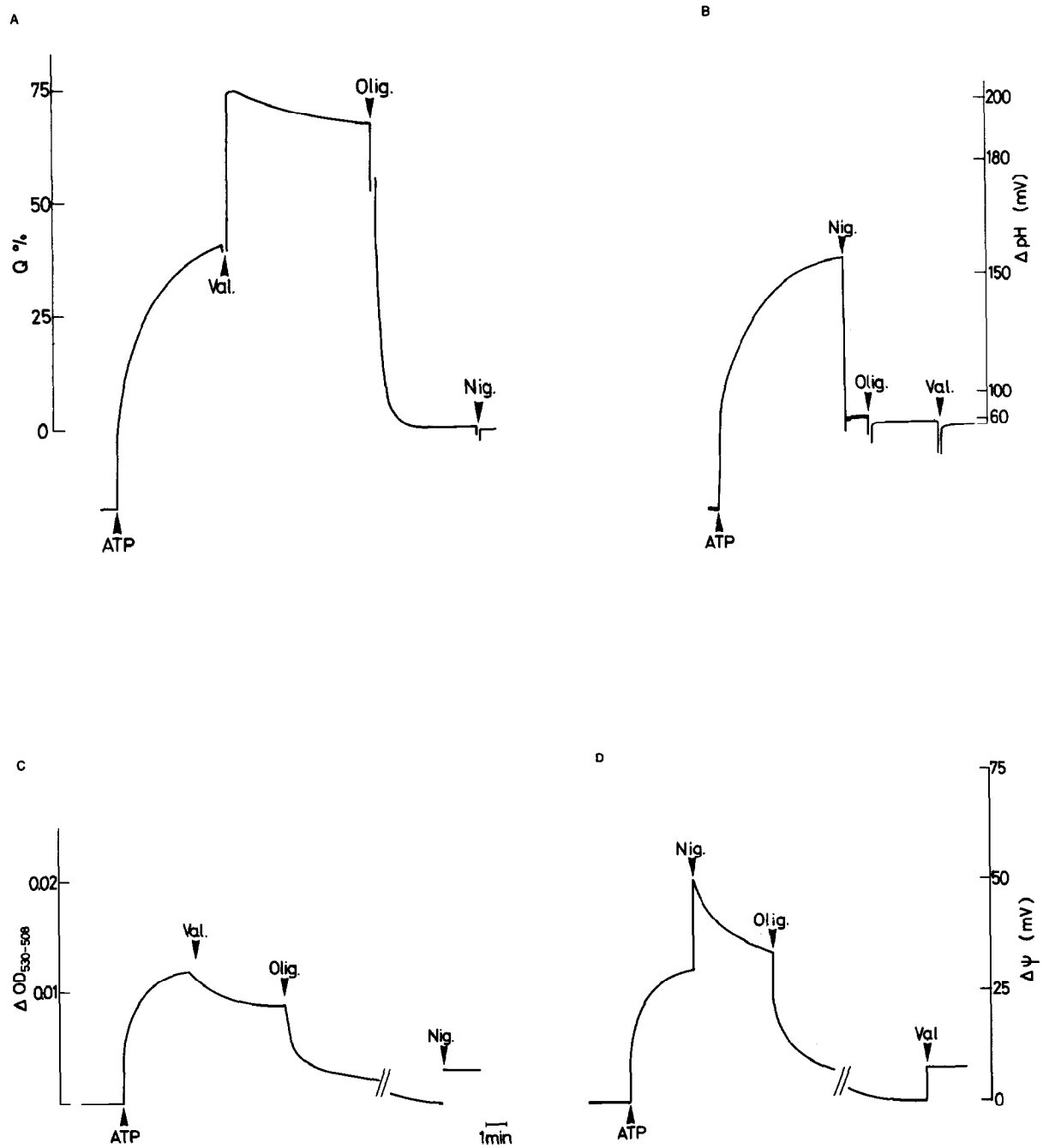

Fig.1. The formation of an electrochemical potential difference of protons coupled to ATP hydrolysis. The traces show the effect of the ionophorous antibiotics and of oligomycin in different sequences of addition, on ΔpH (A and B) and on Δψ (C and D) induced in the dark by ATP, as monitored with 9-aminoacridine fluorescence and carotenoid band shift, respectively. The medium for the assay contained: Na-glycylglycine buffer, pH 8.5, 64 mM; KCl, 50 mM; $MgCl_2$, 2 mM; Na_2HPO_2, 2 mM chromatophores corresponding to 36 μg bacteriochlorophyll. Additions: 9-aminoacridine 5 μM; ATP 1 mM; nigericin 4 μg/ml; valinomycin 4 μg/ml; oligomycin 5 μg/ml.

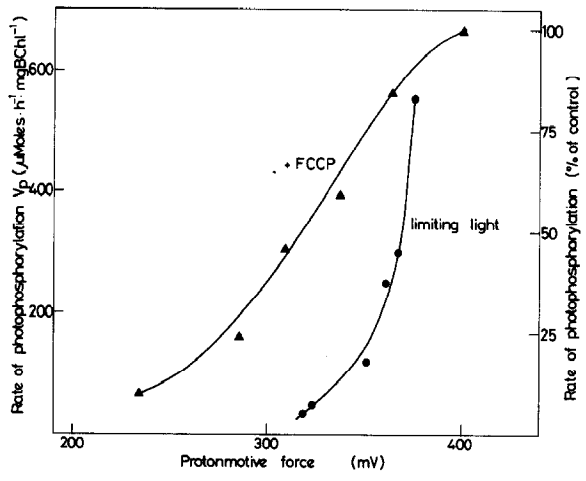

Fig.2. The relationship between the rate of photophosphorylation and the extent of Δp under conditions of partial uncoupling and limiting light intensity in highly active chromatophores. The concentration of FCCP varied from 1.10^{-7} to 2.10^{-6} M (▲). The intensity of the indident actinic light was changed from 1.2×10^6 to 5.6×10^4 ergs. cm^{-2}. s^{-1} by means of steel-net screens or neutral density filters (●).

phorylation and the extent of Δp in highly active chromatophores (665 μmol. h^{-1}. mg Bchl^{-1}) is shown in fig.2. The behaviour of this preparation is quantitatively similar to that already published for lower phosphorylating vesicles [2], namely the addition of the uncoupler FCCP progressively decreases both v_p and Δp, while the limitation of electron flow, obtained by diminishing the intensity of actinic light, hardly affects Δp but exerts a strong inhibition on v_p. The results of these experiments make unlikely the interpretation suggested above, that the differential behaviour of v_p versus Δp, in response to uncouplers or to limited electron flow, is mainly due to the heterogeneity of the vesicle population. To clarify further this contention, the behaviour of membranes in which photophosphorylation had been diminished by means of two independent approaches has been examined.

Sonication of bacterial chromatophores, suspended in 1 mM EDTA, has been shown to decrease largely the activity of photophosphorylation by specifically detatching the extrinsic portion of the ATP synthetase [15]. In agreement with reported data, this treatment in *Rps. capsulata* does not impair drastically the light-dependent active proton uptake [16] or the formation of a protonic potential difference across the membrane [13]. This technique allows control of the number of active ATP synthetase molecules present on the membrane [17]. In EDTA-sonicated chromatophores of *Rps. capsulata*, strain Kbl, whose phosphorylation activity had been lowered to a value corresponding to about 10% original (i.e., from 665–62 μmol. h^{-1}. mg Bchl^{-1}), some decrease of Δp, in both of its components, is observed (fig.3). The overall protonmotive force amounts to 344 mV. This observation indicates that EDTA extraction has some limited effect on the extent of the gradient, on a quantitative basis, in analogy with that observed in other phosphorylating systems [18–21]. In this resolved preparation the discrepancy between the response of v_p versus Δp to the addition of uncouplers or to limitation of light intensity, is again very marked and comparable with that observed in highly active membranes.

Treatment with DCCD has been the second approach taken for limiting the capability of ATP synthesis in chromatophores. This compound is known to bind in many systems specifically to a hydrophobic subunit of ATP synthetase [22] with a stable covalent bond [23] and to improve the capability of the membrane in energy conservation [18], possibly by blocking specifically a proton pore [24]. Also in

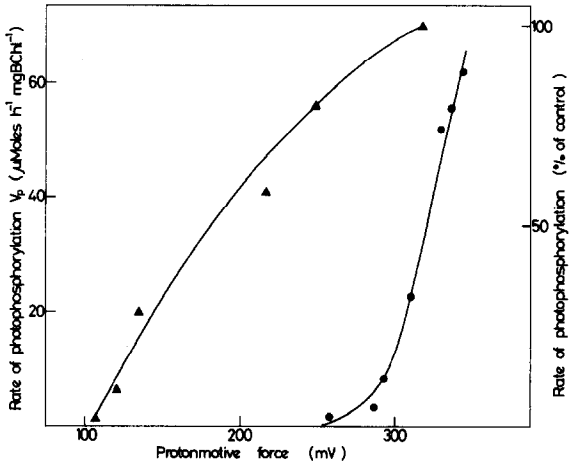

Fig.3. The relationship between the rate of photophosphorylation and the extent of Δp under conditions of partial uncoupling and limiting light intensity in EDTA-sonicated chromatophores. Conditions as in fig.2.

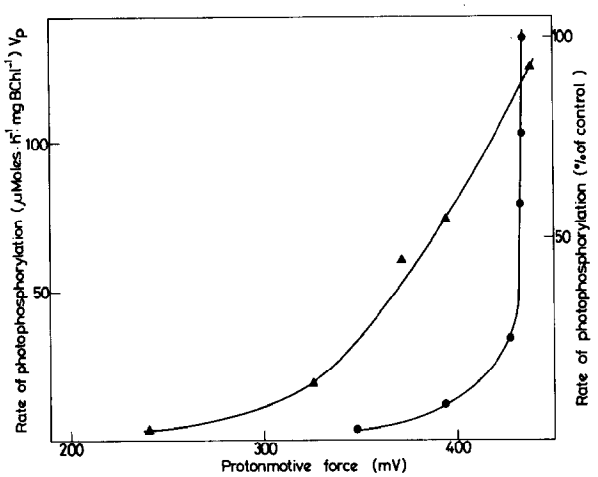

Fig.4. The relationship between the rate of photophosphorylation and the extent of Δp under conditions of partial uncoupling and limiting light intensity in DCCD-treated chromatophores. Conditions as in fig.2.

Rps. capsulata chromatophores DCCD acts as a powerful energy transfer inhibitor, decreasing drastically ATP synthesis and hydrolysis and stimulating the extent of the protonic gradient; treatment of chromatophores with DCCD, at a concentration of 160 nmol. mg $Bchl^{-1}$ (6.9 nmol/mg protein), results in a comparable inhibition, by about 80%, of photophosphorylation and of ATPase activity; a significant stimulation of the extent of ΔpH (from 3.0–3.3 units) and of its rate of onset has also been observed.

The behaviour of DCCD-treated chromatophores is shown in fig.4; the usual difference in pattern of v_p versus Δp in uncoupled and electron flow-limited membranes is still observed. Particularly noticeable is the response of this preparation to the limitation of light intensity: a 4-fold decrease in the rate of ATP synthesis does not correspond to any change in the extent of Δp.

4. Conclusions

Based on the average size of a photosynthetic unit (100 molecules Bchl/reaction center) and the turnover time of the cyclic electron transport system (of the order of 10 ms [25]), the rate of photophosphorylation observed in the preparations used in these studies (600–900 μmol. h^{-1}. mg $Bchl^{-1}$) would correspond to a theoretical ATP/$2e^-$ value of 0.36–0.55. This indicates that a large percentage of photosynthetic units are associated with an ATP synthetase complex. The interpretation that the differential response of v_p versus Δp towards addition of uncoupling agents or limitation of electron flow is due to the presence of a large proportion of non-phosphorylating chromatophores (each containing av. 50 photosynthetic units [26]) appears therefore untenable. Further support to this conclusion is afforded by the results obtained with chromatophores partially depleted of coupling factor or inhibited by DCCD, which behave in this respect similar to the native particles.

The extent of the carotenoid signal and of the quenching of 9-aminoacridine is highly resistant to the limitation of light intensity, indicating a highly effective onset of protonmotive force when only a limited number of photosynthetic units are photoactivated. This may suggest a high degree of negative cooperativity between photosynthetic units of a single chromatophore, consistent with the properties of a delocalized membrane potential and transmembrane pH difference [27] of which these spectroscopic signals are thought to be quantitative indicators [2,9]. This behaviour is on the other hand opposite to what expected if a localized short range interaction (dipole–dipole interaction for carotenoids and electrostatic binding for acridines) were the basis of these phenomena. However, the high cooperativity characteristic for the formation of Δp, cannot be demonstrated for the coupling between photosynthetic electron transport and photophosphorylation, as would be expected if production of a protonic electrochemical potential difference were the necessary and sufficient condition for ATP synthesis. That the low quantum yield of photophosphorylation as compared to formation of Δp is not due to heterogeneity of the chromatophore population, but possibly to short-range functional interactions between electron transport chains and ATP synthetase complexes, seems to be clearly established by the above data. Results pointing to similar conclusions have been obtained also with mitochondrial systems [28,30] (see however opposite results obtained in chloroplasts [11,31]).

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