ENERGY TRANSDUCTION IN PHOTOSYNTHETIC BACTERIA
V. Role of Coupling Factor ATPase in Energy Conversion as Revealed by Light or ATP-induced Quenching of Atebrine Fluorescence

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1. Introduction

The quenching of atebrine fluorescence has been shown to change with changes in the energetic state of phosphorylating membrane systems [1–3]. More recently it has been suggested that this phenomenon is related to the distribution of atebrine across the membrane, in response to the formation of a transmembrane pH gradient [4], and that the mechanism of atebrine uptake is similar to that previously proposed for uptake of other amines by illuminated chloroplasts [5].

In recent work on chromatophores from *Rhodopseudomonas capsulata* a protein coupling factor has been isolated which is required for photosynthetic [6] and respiratory [7, 8] phosphorylation, oligomycin sensitive ATPase and ATP-$^{32}P_i$ exchange, but not for electron flow in extracted membranes. The detachment of this protein causes a marked decrease in ATP synthesis and related partial reactions, but has practically no effect on light-induced proton uptake [9]. These findings might indicate that the coupling factor is not involved in energy conservation but is required for energy transduction. In this paper we present evidence based on light or ATP-driven quenching of atebrine fluorescence which gives further support to this hypothesis, and proves that the function of *Rps. capsulata* coupling factor is in the terminal step of ATP synthesis.

2. Materials and methods

Rhodopseudomonas capsulata, strain St. Louis (American Type Culture Collection 23782) was grown anaerobically in the light in the medium described by Ormerod et al. [10].

Phosphorylating and non phosphorylating (EDTA sonicated) particles were prepared according to procedures previously reported [11]. The coupling factor was extracted with 0.05 M Tris Cl pH 7.4, containing 4 mM ATP, from an acetone powder of phosphorylating chromatophores and the purification was carried on as detailed in [11]. Light induced phosphorylation and ATPase activities were measured by conventional methods [11].

Fluorescence was measured at a 90° angle with a filter fluorimeter of conventional design; the excitation light was selected by a Wratten Kodak no. 36 and a Corning 9782 glass filter. The PX 1016 Philips photomultiplier was shielded with 1 cm of saturated $CuSO_4$ solution and a Wratten Kodak no. 58 filter. Actinic light (55 W quartz–halogen bulb) was screened with a Wratten no. 88 A filter.

3. Results and discussion

Light induced quenching of atebrine fluorescence

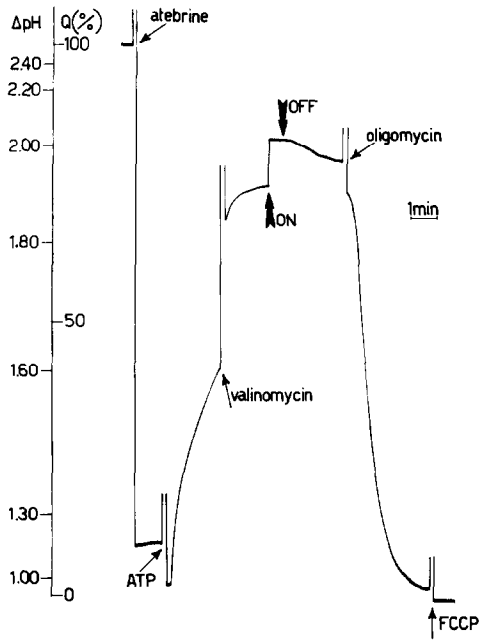

Fig. 1a.

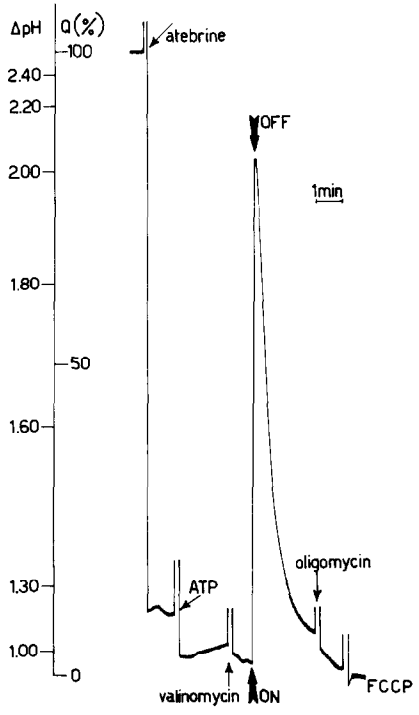

Fig. 1b.

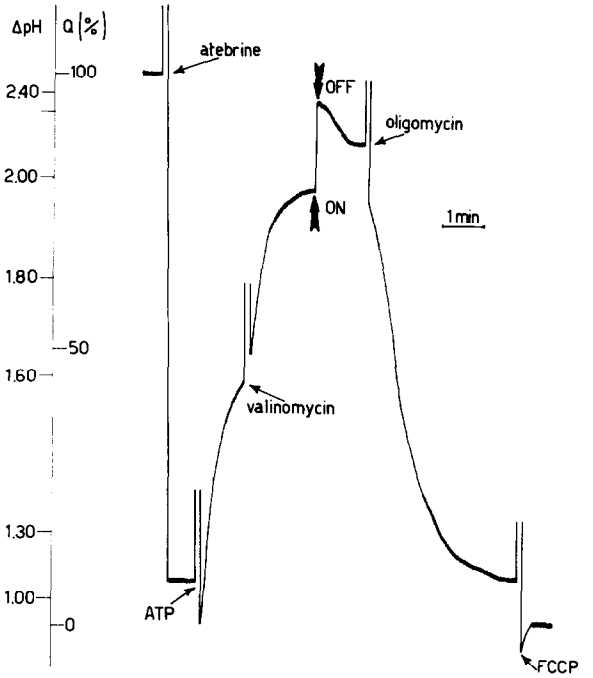

Fig. 1c.

Fig. 1. Quenching of atebrine fluorescence driven by ATP and light in *Rps. capsulata* photosynthetic membranes: a) untreated particles (25 μg BChl.); b) EDTA sonicated particles (30 μg BChl.); c) EDTA sonicated particles (30 μg BChl.) reconstituted with 22 μg coupling factor protein.

The assays contained in a final vol. of 2.5 ml: phosphate buffer, pH 7.8, 32 mM; $MgCl_2$, 5 mM; Na succinate, 0.2 mM; KCl, 100 mM and particles as indicated. Other additions were: atebrine, 0.8 μM; ATP, 2 mM; valinomycin, 0.8 μg/ml; oligomycin, 4 μg/ml; FCCP, 1 μM.

In the reconstitution experiment (fig. 1c), particles and 22 μg of coupling factor were incubated at 30° in a volume of 0.60 ml containing 33 mM phosphate buffer, pH 7.8 and 5 mM $MgCl_2$. After 12 min the incubation mixture was transferred to the fluorescence cuvette containing all other components of the assay to give the final concentrations indicated above.

The pH scale was calculated assuming that atebrine distributed as an ideal diamine with pK's 7.9 and 10.1 [4] in an assumed internal volume of 100 μl/mg BChl (A.R. Crofts [18] and R.J. Codgell, unpublished observation).

Table 1
Restoration of ATP driven quenching of atebrine fluorescence, photophosphorylation and ATPase activity by increasing amounts of purified coupling factor.

Conditions	Fluorescence quenching (%)			Photophosphorylation (μMoles ATP/hr mg BChl.)	ATPase activity (μMoles P_i hydrolized/ hr mg BChl.)	
	ATP	ATP + valinomycin	Light		Total	Oligomycin sensitivie
EDTA-sonicated particles	0	0	94	24.5	13.8	7.0
plus 5.5 μg coupling factor	21	–	94	58.2	26.8	17.6
plus 11 μg coupling factor	28	37	93	86.2	35.6	23.8
plus 22 μg coupling factor	52	79	94	90	47.6	25.2
plus 110 μg coupling factor	71	85	93	107	–	–

Reconstitution experiments were performed essentially as described in fig. 1, except that glycylglycine buffer, pH 8, 66 mM and Tris acetate pH 8, 100 mM were used for measuring photophosphorylation and ATPase activities, respectively.

by *Rps. capsulata* chromatophores resembles in its characteristics the phenomenon previously described in other systems. The steady state level of quenching in saturating light is dependent upon the amount of membrane added but reaches a maximum around 90 to 95%. The light response is sensitive to detergents (Triton X-100, 0.04% v/v), uncouplers (FCCP, 1 μM) and electron transport inhibitors (Antimycin A, 1.5 μM) and is inhibited by nigericin type antibiotics and ammonium salts. Valinomycin plus K^+ stimulates the rate of onset, and less markedly the steady state level of quenching. These observations, as a whole, confirm that light-induced quenching of atebrine fluorescence is dependent upon an energized state of the membrane driven by electron flow and insensitive to oligomycin. More specifically, the characteristics of the response in the presence of ionophorous antibiotics or ammonium salts indicate that atebrine distributed between the inner and outer compartment of the membrane vesicles, at least qualitatively, in response to a pH gradient rather than a membrane potential. In accordance with these conclusions, atebrine, at relatively low concentrations (5.6 to 56 μM), was observed to stimulate the rate and extent of Xenon-flash induced carotenoid band shift, without markedly accelerating the rate of decay.

The energized state of the membrane could also be induced by addition of ATP in the dark. In the experiment shown in fig. 1a, ATP (at a final concentration of 2 mM) was added to coupled photosynthetic membranes, endowed with a high photophosphorylating capability (182 μmoles/hr mg BChl.). After a slight increase of fluorescence due to a nonspecific, membrane independent interaction between ATP and atebrine, a slow quenching phenomenon was observed, which reached a steady state level after about 2 to 3 min. Subsequent addition of valinomycin further stimulated the quenching, confirming the electrogenic mechanism of ATP driven formation of the H^+-gradient. Illumination of ATP energized particles enhanced the quenching to the level observed in the absence of ATP; the light effect was reversed in the dark and fluorescence returned to the previous level. On addition of oligomycin the quenching decreased to that of the de-energized state and further additions of FCCP or Triton X-100 had negligible effect.

When a similar experiment was performed with EDTA-sonicated particles, depleted of coupling factor, the results obtained were markedly different (fig. 1b). With such a preparation ATP was ineffective in promoting an energized state, and valinomycin caused an enhancement of fluorescence, rather than a quenching, possibly due to an electrophoretic efflux of protons driven by the diffusion potential of the K^+ gradient. On the other hand illumination of non phosphorylating membranes elicited a quenching phenomenon which in rate and extent was indistinguishable from that obtained with phosphorylating particles in the absence of ATP. In the dark the quenching was completely reversed and the rate of decay was insensitive to addition of oligomycin.

The effects of ATP and of valinomycin were

nearly completely restored if EDTA-sonicated particles were incubated with purified coupling factor (fig. 1c). At saturating levels of this protein a steady state extent of atebrine fluorescence quenching in the presence of ATP and valinomycin as high as that in the native phosphorylating particles could be obtained. Again ATP driven quenching was completely inhibited by oligomycin. When the membranes were preincubated with non saturating amounts of coupling factor the steady state level of ATP promoted fluorescence quenching increased in parallel with the restored photophosphorylation and oligomycin sensitive ATPase (table 1).

Qualitatively similar results were obtained measuring the quenching of 9-amino-acridine fluorescence, though the latter method gave larger values for ΔpH.

It is therefore evident that non phosphorylating membranes depleted of coupling factor ATPase retain their ability for energy conservation. In fact all functions so far examined, which are linked to a light-induced energized state of the membrane, are still present in non phosphorylating particles. These functions include, in addition to the atebrine fluorescence quenching described above, proton uptake [9], reduction of NAD^+ from succinate and light induced carotenoid spectral shift (Melandri and Cogdell, unpublished observations). The lack of dependence of energy conserving ability of *Rsp. capsulata* chromatophorus upon the presence of coupling factor ATPase is a remarkable feature among energy transducting membranes. In fact all respiratory or photosynthetic systems so far studied have shown a marked loss of energy linked functions promoted by electron flow following the detachment of ATPase (cf. [3, 12–15]). The data presented above also differ significantly from the recent results obtained in *Rhodospirillum rubrum* chromatophores by Johansson et al. [16]. They observed a general decrease of both light-induced and ATP or pyrophosphate induced functions, following the removal of coupling factor using a similar procedure. These functions were partially restored by addition of a crude sonicate fluid, indicating a partial "structural" effect of coupling factor in *Rh. rubrum* system.

In contrast to the effect described above, the coupling factor ATPase is an obligate requirement for ATP induced energization of the membrane. The catalytic function of this protein has therefore to be sought specifically in the terminal phosphorylation reaction leading to ATP synthesis or to ATP utilization in energy driven functions of the membrane.

It has also been demonstrated here that oligomycin completely inhibits membrane energization induced by ATP hydrolysis; this is in agreement with the complete sensitivity of photophosphorylation to this antibiotic. Native or reconstituted ATPase activity of *Rps. capsulata* is however only partially sensitive to oligomycin, though both oligomycin sensitive and insensitive activities are most probably catalyzed by the same coupling factor [17]. These experiments demonstrate therefore that oligomycin insensitive ATPase is unable to transfer energy to the membrane, and confirm that ATPase sensitivity to oligomycin can be considered as an unambigous test for the intactness of the energy transducing multienzyme complex of *Rps. capsulata* membranes.

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