*Biochimica et Biophysica Acta* 808 (1985) 180–185
Elsevier

BBA 41777

# Efficiency of light conversion in photophosphorylation measured in chromatophores fused with liposomes and treated with inhibitors

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(Received November 16th, 1984)
(Revised manuscript received March 22nd, 1985)

Key words: Electron transport; Liposome; Photophosphorylation; Membrane fusion; $H^+$-ATPase; *(Rps. capsulata)*

**Photophosphorylation activity under saturating continuous illumination was estimated in differently treated chromatophores of a B800–850 less mutant of *Rhodopseudomonas capsulata*. The activity was decreased when the chromatophores were fused with liposomes or when inhibitors of the $H^+$ ATPase or electron transport were added. Estimation of the irradiance needed for half-maximal turnover (apparent $K_m$ for light) revealed that fused membranes and inhibitor-treated chromatophores behaved oppositely. In fused membranes the $K_m$ value was increased 5-fold compared to the control chromatophores, from which we conclude that part of the input energy is dissipated and lost for photophosphorylation.**

**Partial inhibition of $H^+$ ATPases or especially of electron transport resulted in a considerable decrease in the $K_m$ value. To explain this, we define a photosynthetic chain as the minimum functional unit being able to convert light energy into ATP. The decrease of the $K_m$ value indicates that photosynthetic chains are not separated from each other in the membrane and exchange at least one of the intermediates, ubiquinone, cytochrome *c*, or protons.**

## Introduction

ATP formation at energy-transducing membranes is driven by a linear or cyclic electron-transport chain [1]. It is generally believed that a close interaction between electron transport complexes must occur to ensure efficient electron flow [2]. Taking into account the lateral mobility of electron carriers, interaction could occur by a collisional mechanism, by stable contact between redox components, or via diffusion of electron carriers [3,4].

In photosynthetic bacteria the cyclic electron transport is driven by reaction centers which are surrounded by antennae-BChl complexes. Two different antennae complexes are present in *Rhodopseudomonas capsulata* and *Rhodopseudomonas sphaeroides*. The light harvesting complex B870 which has a fixed ratio of 25 mol BChl per mol reaction center [5], and the B800–850 complex which is present at different concentrations depending on culture conditions. The B800–850 complex probably connects different reaction center B870 units in fully pigmented cells [6–8].

The hypothetical minimal functional unit which is able to convert light energy into ATP will be called in the following a 'photosynthetic chain'

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Abbreviations: UQ, ubiquinone; BChl, bacteriochlorophyll; FM, fused membranes; M, non-fused membranes; $P_i$, inorganic phosphate; Cyt, cytochrome; Tricine, *N*-[2-hydroxy-1,1-bis (hydroxymethyl)ethyl]glycine.

(analogous to 'respiratory chain'). We are interested in the interaction of the complexes building photosynthetic chains, their stoichiometry, and how the cell optimizes its photosynthetic system. The question will be raised whether the photosyntehtic chains act as being isolated or communicate via one or more 'substrates'. Interaction of different reaction center-B870-units via exciton transfer is severely reduced if the units are separated in the membrane in vivo, as under specific culture conditions, as in a certain membrane fraction, or as in some mutants [6–9]. Interaction on the level of electron-transport complexes has been studied in mitochondria [3,10,11] and photosynthetic bacteria [12,13] by phospholipid enrichment. Dilution of UQ in the plane of the membrane appeared to be only one factor responsible for lowered electron-transport rates because addition of UQ only partially restored photophosphorylation [13]. The chemiosmotic hypothesis [1,14,15] predicts that the electrochemical proton gradient is the free-energy intermediate between electron transport and ATP formation. According to this hypothesis the proton gradient is delocalized over the entire membrane, and can be used by each $H^+$ ATPase of the cell. But in recent years the existence of local interactions between electron-driven proton pumps and $H^+$ ATPases was discussed [4,16,17,18]. These local interactions would restrict the exchangeability of protons between different photosynthetic chains.

Light-saturation curves of photophosphorylation, which represent a tool to investigate maximal capacity and light requirement of the photosynthetic system [19], were applied in the following to investgate these interactions. Chromatophores were isolated from *Rps. capsulata* strain NK3, a mutant lacking B800–850 [20]. This mutant was chosen because photophosphorylation rates after lipid enrichment were more stable than in membranes derived from wild-type cells.

## Materials and Methods

*Rhodopseudomonas capsulata* NK3 [20] was grown in the medium as previously described [21]. Chromatophores were prepared after breaking the cells by French pressuring in 50 mM Tricine buffer (pH 7.8), containing 2 mM magnesium acetate. Liposomes of phosphatidyl choline (Sigma, Type IIs) were prepared in the same buffer by sonication of the phospholipid suspension (100 mg/ml) for 10 min at 5°C.

The fusion of chromatophores and liposomes was carried out by the freeze-thaw technique [22]. Membrane fractions were separated by centrifuging them on a sucrose density gradient (7.5–60% sucrose, w/w) for 15 h at 36000 r.p.m. in a SW41 rotor (Beckman Instruments, Munich). Control membranes were prepared by freezing and thawing fresh chromatophores in the absence of liposomes. They were also separated by density gradient centrifugation. Three vesicle fractions with different buoyant densities were isolated and concentrated by a second ultracentrifugation.

Photophosphorylation was performed as previously described [19] in a medium comprising 100 mM glycylglycine buffer (pH 7.75)/10 mM magnesium acetate/0.1 mM dithioerythreitol/0.1% bovine serum albumin/2 mM $K_2HPO_4$/0.25 mM AMP/0.05 ADP/1.5 units per ml myokinase/3 mM KCN. Membranes were used corresponding to less than 5 $\mu$M BChl to achieve uniform irradiance over the whole sample and to avoid scattering artifacts.

Myokinase was allowed to establish equilibrium between all adenosine nucleotides. The sample was irradiated with continuous monochromatic light ($\lambda = 846$ nm, interference filter NAL 846, Schott, Mainz). The luminescence increase showed that photophosphorylation rates were constant during the time of irradiation. Depending on the irradiance and on the activity of the sample, irradiation times were chosen between 5 and 30 s to obtain comparable ATP levels. In the following dark period (2 min), the luminescence was observed to fall to (or below) the original level. Oligomycin-treated samples were preincubated for 60 min at room temperature, followed by a few light/dark cycles. No preincubation was needed when using venturicidin or antimycin A. The photophosphorylation activity of the sample decreased between 10% and 30% with a constant rate during the light titration. To compensate this loss, two consecutive light titrations were carried out. First, the activities were estimated with increasing and after this with decreasing irradiance values. Finally, the mean was taken. Before each inhibitor addition a fresh sample was prepared. A Woolf plot [23] was used

to derive maximum photophosphorylation ($V_{max}$) and the irradiance needed for half-maximal turnover (apparent $K_m$).

Protein was determined by the Lowry procedure [24]. $H^+$ ATPase activity was measured in 50 mM Tris-HCl (pH 8.0) buffer containing 3 mM magnesium acetate and 2 mM ATP. Phosphate released was analyzed according to Taussky and Schorr [25].

## Results

Fusion of chromatophores with liposomes resulted in vesicles of lower density than the original chromatophores. The membrane material was resolved into three visible bands, having buoyant densities of 1.1513, 1.1059 and 1.0766 $g \cdot cm^{-3}$. We used the material from the band with the lowest density to measure $H^+$ ATPase activity and photophosphorylation rates. This fraction, henceforth called FM, showed the lowest photophosphorylation activity. Its rate (2–5 μmol ATP/mg protein per h) was substantially lower than the rate found in non-fused control chromatophores (30 μmol ATP/mg protein per h), henceforth called M.

The loss in photophosphorylation activity could be partially restored by addition of $UQ_{10}$ [13]. The specific $H^+$ ATPase activity found in fraction FM (5 μmol $P_i$/mg protein per h) was less than half of that found in fraction M (13 μmol $P_i$/mg protein per h). This decreased ATPase activity probably reflects loss of $H^+$ ATPases during the fusion process or a lower specific activity due to the changed lipid environment [26].

The maximum rate of photophosphorylation ($V_{max}$) decreased similarly in fractions M and FM with increasing amounts of antimycin A (Fig. 1a and b). Addition of oligomycin lowered $V_{max}$ by decreasing the number of active $H^+$ ATPase complexes (Fig. 1c and d). Similar results were obtained using venturicidin (data not shown). Increasing the concentration of antimycin A after addition of oligomycin further reduced $V_{max}$ (Fig. 1c and d). Partial inhibition of photophosphorylation with antimycin A, oligomycin, or a combination of both produced a photosynthetic system in fused membranes which showed different light saturation behavior, with $K_m$ values between 9.5

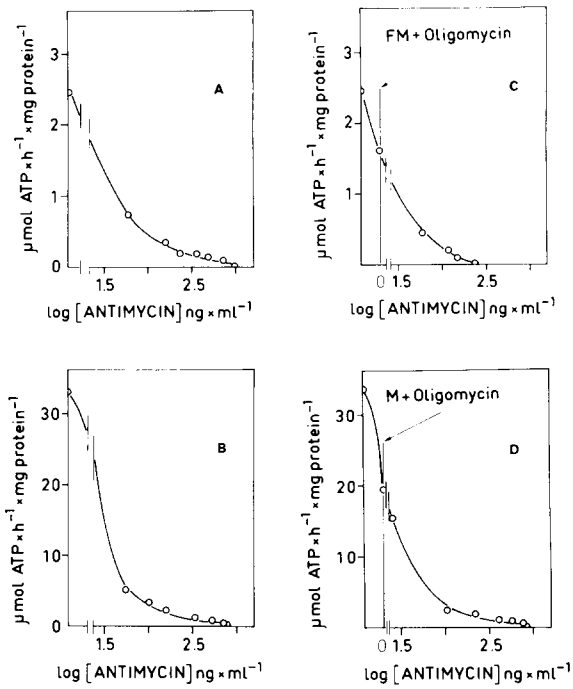


Fig. 1. Titration of photophosphorylation activity ($V_{max}$) with increasing concentrations of antimycin A. The samples were chromatophores fused with liposomes (fraction FM) or chromatophores (fraction M). Protein concentrations 290 μg/ml (fraction FM) and 240 μg/ml (fraction M). (a) Fraction FM without oligomycin; (b) fraction M without oligomycin; (c) fraction FM with oligomycin (22 ng/ml); (d) fraction M with oligomycin (43 ng/ml).

and 60 $W/m^2$ (Fig. 2). Similar results were obtained using control membranes (not shown).

The $K_m$ as a function of antimycin A concentration decreased steadily with increasing inhibitor concentration, finally reaching a value 5-times lower than without inhibitor. Fused and non-fused membranes showed a similar behaviour except that the $K_m$ of fraction FM generally was 4-times higher than the $K_m$ of fraction M (Fig. 3a and b).

Addition of oligomycin also reduced the $K_m$, and subsequent titration with antimycin A resulted in a further steady decrease with increasing inhibitor concentration (Fig. 3c and d). Similar results were obtained with venturicidin (data not shown).

The $K_m$ observed in the presence of inhibitors did not pass below a value of about 4 $W/m^2$ (Fig. 3b and d). The same value was found in untreated

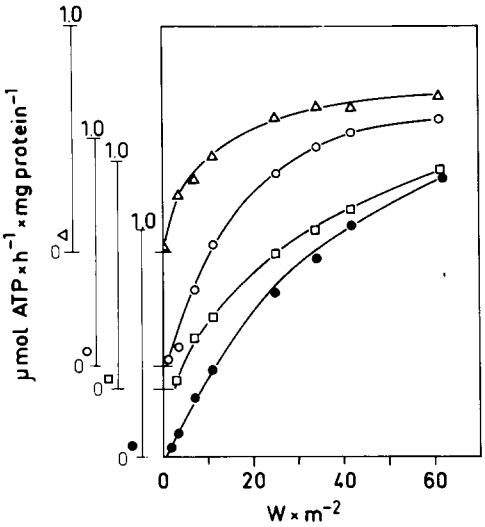


Fig. 2. Light saturation curves of photophosphorylation. Fraction FM under different inhibitor conditions. Protein concentration, 290 μg/ml. ●, Fraction FM without additions; bar, 1 μmol ATP/mg protein per h with $K_m = 60$ W/m$^2$; □, fraction FM with 22 ng/ml oligomycin; bar, 1 μmol ATP/mg protein per h with $K_m = 43$ W/m$^2$; ○, FM with 60 ng/ml antimycin A; bar, 0.5 μmol ATP/mg protein per h with $K_m = 20$ W/m$^2$; △, fraction FM with 24 ng/ml oligomycin and with 150 ng/ml antimycin A; bar, 0.2 μmol ATP/mg protein per h with $K_m = 9.5$ W/m$^2$.

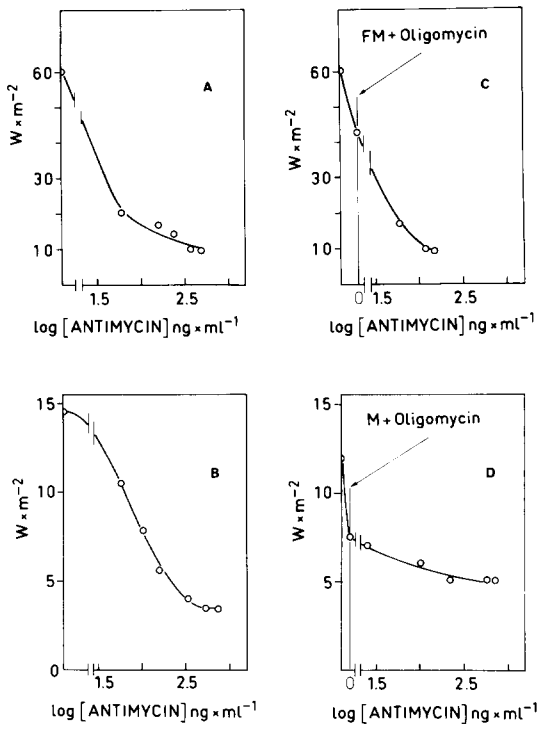


Fig. 3. Dependence of the apparent $K_m$ for light on antimycin A concentration. Samples and conditions as described in Fig. 1.

membranes derived from wild-type cells which have become optimized to utilize extremely low irradiances [34].

## Discussion

The response of fused and control membranes to inhibitors of electron transport and of $H^+$ ATPases or a combination of both was similar (Fig. 1).

Lipid enrichment increased the $K_m$ value for light, whereas inhibitor addition decreased it (Figs. 2 and 3). The increased $K_m$ in fused membranes indicates that the system needs more light to synthesize ATP. We cannot exclude that part of the increase originates from higher light scattering in fraction FM compared to fraction M, but we do not regard this effect to be dominant because the protein and lipid concentrations were kept so low that a transparent sample was obtained. Furthermore, the exciting light had a long wavelength, so scattering was lower than in visible light.

Since it was shown that low detergent concentrations applied to solubilize the membranes did not disrupt B870 reaction center units [27], it can be assumed that lipid introduction did not separate these complexes. Accordingly, exciton-trapping rates should not be different in fractions FM and M, and the increased need for light probably reflects a higher dissipation of input energy in fused that in non-fused membranes. This

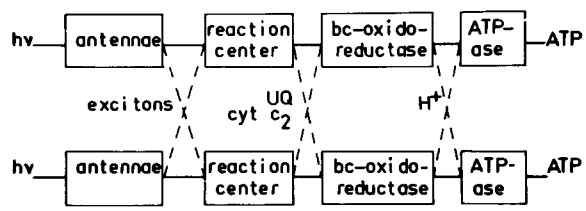


Fig. 4. Scheme for possible substrate exchanges between two adjacent photosynthetic chains.

leak could be located either at the level of free-electron carriers or at the level of protons.

How can the shift in $K_m$ after the addition of inhibitors be explained? Respiration in mitochondria has been described by a sequential multienzyme reaction in which most of the intermediates are freely diffusible [28]. We want to apply the same formalism to the photosynthetic system.

Two photosynthetic chains (defined in the introduction) eventually can exchange their intermediates, excitons, electron carriers and protons (Fig. 4).

If there would exist no exchange at all, the two chains would behave as being functionally isolated from one another. Inhibition of one ubiquinol Cyt $c_2$ oxidoreductase with antimycin A would stop ATP synthesis totally at this chain, but the other chain would not be influenced. Therefore, the $V_{max}$ of the whole system would become reduced, but not the $K_m$ for light. But if we assume that some intermediates such as UQ, Cyt $c_2$, or excitons are exchangeable, the blocked ubiquinol Cyt $c_2$ oxidoreductase could be 'bypassed'. In this case the number of active antennae and reaction centers would be increased in relation to the ubiquinol Cyt $c_2$ oxidoreductases still active. If non of the reactions located in the chain before the ubiquinol Cyt $c_2$ oxidoreductase are rate limiting, the $K_m$ should be decreased. Actually, the uncertainty about the position of the rate-limiting step [16,29,30] increases the difficulty to determine the intermediate(s) responsible for the shifted $K_m$. It is still an unanswered question if there is one single rate-limiting reaction in a multienzyme chain [31,32]. Indeed, the exchangeability of just protons between the chains likewise could explain the shift in $K_m$ (provided that the $H^+$ ATPases are not rate limiting).

Excitons probably are not involved in this exchange process because (i) no interconnecting B800–850 complexes are present in the chromatophores used and (ii) the shift in $K_m$ also was observed in fused membranes which probably have increased distances between different B870 reaction center units.

The exchangeability of substrate(s) demonstrated here with inhibitors in vitro probably is present also in the bacterial cell in vivo. A multienzyme system could become optimized by altering the ratio of its enzymes [33]. The same optimization could take place with membrane-bound complexes in the photosynthetic system of the growing cell.

## Acknowledgements

The authors wish to thank Mrs. Johanna Nährig for drawing the figures. This work was supported by a grant from the Deutsche Forschungsgemeinschaft. A.F.G. wishes to acknowledge partial support from the CONICET, Argentina.

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