Journal of Bioenergetics and Biomembranes, Vol. 11, Nos. 1/2, 1979

The Stimulation of Photophosphorylation and ATPase by Artificial Redox Mediators in Chromatophores of *Rhodopseudomonas capsulata* at Different Redox Potentials[1]

Assunta Baccarini-Melandri, B. Andrea Melandri, and Günter Hauska

Institute of Botany, University of Bologna, Bologna, Italy and University of Regensburg, FB Biologie und Vorklinische Medizin, Institut für Botanik, GFR

Received January 20, 1979

Abstract

(1) Inhibition of cyclic phosphorylation in chromatophores of *Rhodopseudomonas capsulata* by antimycin A can be fully reversed by artificial redox mediators, provided the ambient redox potential is maintained around 200 mV. The redox mediator need not be a hydrogen carrier in its reduced form, N-methyl-phenazonium methosulfate and N,N,N′,N′-tetramethyl-*p*-phenylenediamine being equally effective. However, the mediator needs to be lipophilic. Endogenous cyclic phosphorylation is fastest around 130 mV. A shift to 200 mV can also be observed if high concentrations of artificial redox mediator are present in the absence of antimycin A. (2) ATPase activity of *Rhodopseudomonas capsulata,* in the light as well as in the dark, activated or not activated by inorganic phosphate, can also be stimulated by N-methylphenazonium methosulfate. This stimulation is highest at redox potentials between 60 to 80 mV and is sensitive to antimycin A. In this case N,N,N′,N′-tetramethyl-*p*-phenylenediamine is much less effective.

[1]Abbreviations: PES, N-methyl-phenazonium ethosulfate; PMS, N-methyl-phenazonium methosulfate; TMPD, N,N,N′,N′-tetramethyl-*p*-phenylenediamine; DAD, diaminodurene (2,3,5,6-tetramethyl-*p*-phenylenediamine); Bchl, bacteriochlorophyll; FCCP, carbonylcyanide-*p*-trifluoromethoxy-phenylhydrazone; E_h, redox potential; E_m, midpoint redox potential.

0145-479X/79/0400-0001$03.00/0

Introduction

Cyclic photophosphorylation of *Rhodospirillum rubrum* (1, 2) and *Rhodopseudomonas capsulata* (3) is inhibited by antimycin A, but only with the former has an appreciable restoration by artificial redox compounds been observed (2–5). It is noteworthy that both PMS, a hydrogen carrier, and TMPD, an electron carrier (6), are active, in contrast to cyclic phosphorylation in chloroplasts, where a hydrogen carrier is required (7). On this basis possible pathways for artificial cyclic electron flow have been discussed, either comparing chloroplasts and photosynthetic bacteria in general (8), or comparing *R. rubrum* with *Rps. capsulata* (5, 8). However, the necessity of a redox poise for optimal turnover of cyclic electron flow, first described by Bose and Gest (9), and associated photophosphorylation have not been considered in these discussions. E_h optima between 0 and 100 mV for photophosphorylation in chromatophores of *R. rubrum* (10), *Chromatium vinosum* (11), and of *Rps. sphaeroides* (12) have been reported.

ATPase activity is known to be stimulated by artificial redox compounds like PMS in mitochondria (13) and in chromatophores of *R. rubrum* (14). This effect is dependent on the ambient redox potential and has been interpreted either by a stimulation of reversed electron flow (13) or by an interaction of electron transport components with ATPase (14).

In the present paper we show that endogenous phosphorylation in chromatophores of *Rps. capsulata* as well as phosphorylation in the presence of antimycin A and of artificial redox mediators both depend strictly on the ambient redox potential, with markedly different E_h optima. In addition, such a dependence is found for ATPase activity.

Possible pathways for the action of the artificial redox mediators employed are reconsidered, especially in view of the more recent sophisticated schemes for cyclic electron flow in photosynthetic bacteria (15, 16).

Materials and Methods

Rps. capsulata, strain Kb1, was grown under the culture conditions detailed earlier (17), and chromatophores were prepared therefrom according to the procedure described previously (18). Photophosphorylation was carried out at 30°C under strict anaerobic conditions in a specially devised reaction vessel furnished with a Pt-electrode and connected to a calomel reference electrode via a salt bridge. The assay medium contained in a total volume of 2 ml: 120 μmol glycylglycine, pH 8.5, 20 μmol $MgCl_2$, 3 μmol ADP, 60 μg

BChl chromatophores, and redox mediators at the concentrations reported in the captions to the figures; the oxidation reduction potential was adjusted by addition of small amounts of oxidizing or reducing agents (ferricyanide, ascorbate, and NADH) in the light before and after the reaction was started by injection of 5 μmol $^{32}P_i$ (ca. 10^6 cpm) through a rubber septum. After 2 min under controlled redox conditions light was turned off and 1 ml of the reaction assay was quickly mixed with 0.2 ml of ice- cold 25% trichloroacetic acid. Incorporation of $^{32}P_i$ into organic phosphate was measured according to Avron (19). Illumination was provided by a 150-W lamp filtered through a red plastic foil and focused by a water-containing round bottom flask; the intensity of actinic light measured with a Yellow Springs Instrument Radiometer (model 65 A) was 2×10^5 ergs $\cdot$ cm^{-2} $\cdot$ sec^{-1}.

ATPase activity was measured under similar experimental conditions in the dark or in the light. In the latter case a preillumination time of 5 min was necessary to achieve maximal activation of the enzyme (20). The assay mixture contained in a final volume of 2 ml: 120 μmol glycylglycine, pH 8.5, 5 μmol $MgCl_2$, 0.4 μmol EDTA, 2 μmol inorganic phosphate, and 88 μg BChl chromatophores. The reaction was started by addition of 5 μmole γ-labeled (^{32}P)-ATP (ca. 10^6 cpm) and was carried on for 3 min at 30°C. Special assay conditions are detailed in the caption to Fig. 7. The amount of $^{32}P_i$ released was determined according to Horio et al. (21). Bacteriochlorophyll was measured in acetone:methanol extracts according to Clayton (22).

Results

Photophosphorylation

Inhibition of photophosphorylation by antimycin A in chromatophores of *Rhodopseudomonas capsulata* can be reversed by artificial redox compounds, provided that the redox potential (E_h) of the reaction mixture is kept within a narrow, optimal range. Figure 1 shows this for three different redox compounds. PMS and TMPD are about equally effective, while DAD is a poor mediator. The optimal E_h seems to be independent of the midpoint potential (E_m) of the mediator employed—80 mV for PMS, 260 mV for TMPD, and 220 mV for DAD at standard conditions (23)—and is about 200 mV. It is only some 20 mV higher with TMPD than with PMS in the experiment of Fig. 1, although the standard midpoint potential of these two compounds differs by 180 mV. Pyocyanine, with a standard midpoint potential of -38 mV, did not reverse the inhibition by antimycin. As has been observed in chromatophores of *R. rubrum* earlier (6), and in contrast to cyclic

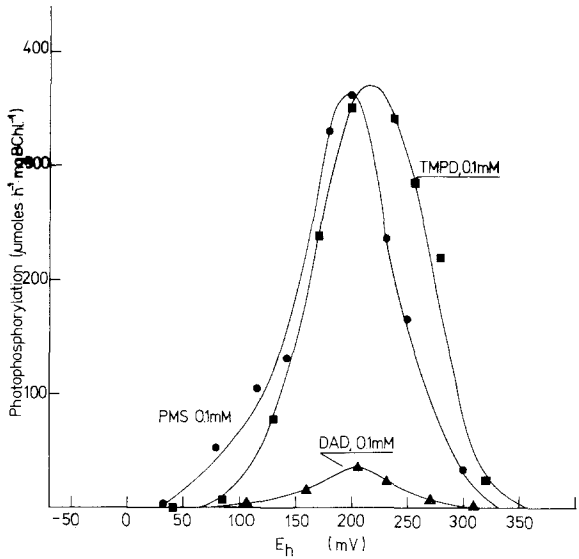

Fig. 1. E_h dependence of cyclic photophosphorylation restored by artificial redox mediators after inhibition by antimycin A. Assays conditions as described under Materials and Methods. The concentration of the redox dyes used was 100 μM; antimycin was present at a concentration of 1 μg/ml. The rate of photophosphorylation, assayed by a standard method (18), was 400 μmol ATP synthesized per hour per mg BChl, and was inhibited 90% by addition of antimycin (1 μg/ml).

phosphorylation in chloroplasts (7, 24, 25), the artificial redox mediator need not be a hydrogen carrier in its reduced form; TMPD, not carrying hydrogen, gives stimulation to the same extent as PMS, a hydrogen carrier. However, as found for chloroplasts (7, 26), the compounds need to be lipophilic; sulfonated PMS, which is lipid-insoluble, is ineffective (data not shown).

Figure 2 shows the titration of this effect with all three compounds at optimal E_h. Saturation is reached at relatively high concentrations only, especially with DAD. PMS shows a tendency to inhibit at high concentration, in contrast to the other two compounds. This is reminiscent of the concentration optimum of PMS in cyclic photophosphorylation with chloroplasts (27). After more than 90% inhibition of cyclic photophosphorylation in chromatophores of *Rhodopseudomonas capsulata* with antimycin A, about 85% restoration with PMS and over 100% restoration with TMPD is obtained. DAD gives restoration to some 30%; this is less than reported before (5).

The optimal E_h is independent of dye concentration. It is always found at 200 mV, as shown for 0.1 and 0.5 mM PMS in Fig. 3. The same E_h optimum

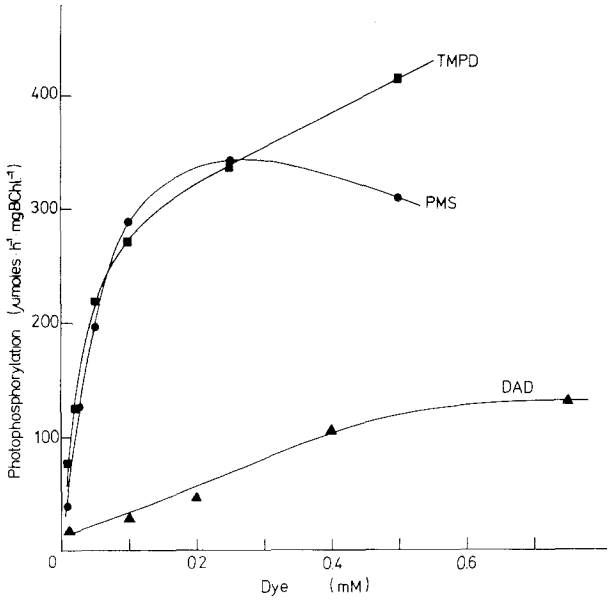

Fig. 2. Dependence of antimycin-inhibited cyclic photophosphorylation, measured at optimal E_h (+200 mV), on the concentration of artificial redox mediators.

is observed if PMS is associated with a mixture of redox dyes at low concentration (9,10-anthraquinone-2-sulfonate, DAD, pyocyanine, PES, 2.5 μM each), which give a better redox poise of the external phase in a broader range (data not shown). The optimum at 200 mV suggests that the rate of restored photophosphorylation is not limited by reduced PMS.

At 10 μM PMS only a small restoration is observed—40 μmol ATP/mg BChl per hour at 200 mV (Fig. 2). Even lower rates are observed with each of the other redox compounds at micromolar concentrations, in the presence of antimycin A. This, in our opinion, provides the possibility to study the E_h dependence of endogenous cyclic photophosphorylation in the absence of antimycin A, employing such low concentrations of redox mediators.

The optimum for endogenous photophosphorylation, either in the presence of a mixture of redox dyes, 2.5 μM each (Fig. 4a), or in the presence of 10 μM PMS (Fig. 4b), lies around 130 mV, which is significantly lower than the optimum for the reversal of antimycin inhibition. At higher concentration of PMS, in the absence of antimycin A, the E_h optimum is shifted to about 200 mV again (Fig. 4a,b), which corresponds to the optimum found in the presence of antimycin A (Figs. 1 and 3). At lower E_h, around 100 mV,

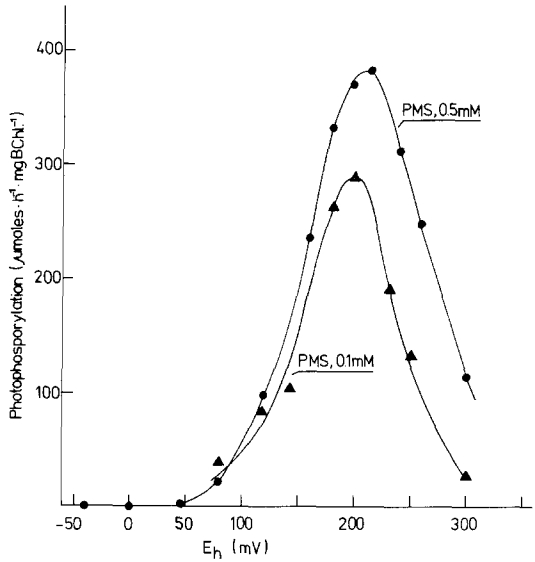

Fig. 3. E_h dependence of photophosphorylation measured in the presence of antimycin, at two different concentrations of PMS.

PMS seems to be inhibitory, while at higher E_h, PMS stimulates, reflecting the competition of the endogenous and the artificial pathway and their different E_h dependence.

ATPase

A dependence of ATPase on E_h is suggested by earlier experiments with chromatophores from *Rhodospirillum rubrum* (14). In Fig. 5 we show this dependence for ATPase of chromatophores of *Rhodopseudomonas capsulata,* measured in the dark and activated by phosphate (28). In contrast to photophosphorylation in the presence of antimycin A (Figs. 1 and 2), ATPase is much more stimulated by PMS than by TMPD, and the optimal E_h is found between 60 and 80 mV. The stimulation at the E_h optimum is about 100% with PMS and only about 30% with TMPD. A stimulation equivalent to that observed in the presence of 0.1 mM PMS and with similar E_h dependence is found with a mixture of redox compounds: PMS, PES, pyocyanine, DAD, and 9,10-anthraquinone-2-sulfonate, 10 μM each (data not shown). As in the case of photophosphorylation, the redox compound has to be lipophilic to stimulate ATPase. Sulfonated PMS is ineffective (26).

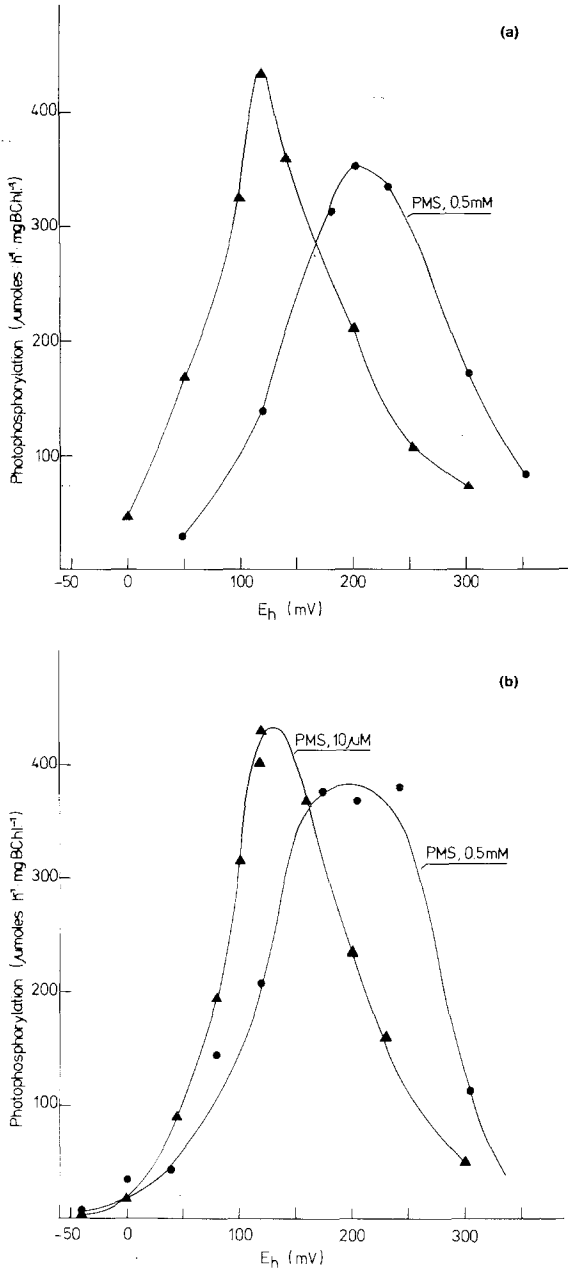

Fig. 4. E_h dependence of photophosphorylation measured in the absence of antimycin. (a) The redox mediators, DAD, PMS, PES, pyocyanine, and juglone, 2.5 μM each, were included in the reaction mixture. (b) Only 10 μM PMS were present. In the experiments represented by the solid circles in (a) and (b), 0.5 mM PMS was present additionally.

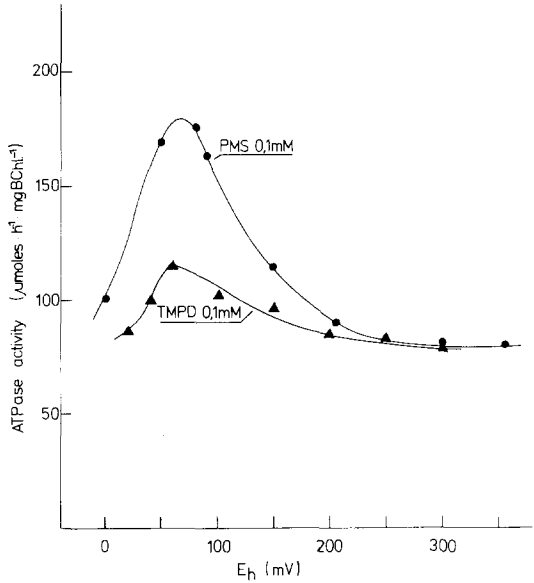

Fig. 5. E_h dependence of ATPase activity, measured in the dark in the presence of P_i (2 mM). The rate of ATPase activity under the same assay conditions, but in the absence of redox mediators, was 80 μmol P_i released per hour per mg BChl.

As seen in Fig. 6 at suboptimal conditions, either in the presence of antimycin A (curve b), or at high E_h (curve c), FCCP exhibits a dual effect on ATPase, as reported before (29). At about 10^6 M it stimulates, but at higher concentrations it inhibits ATPase, reflecting that the active state of the ATPase requires some energization of the membrane (29, 30). At optimal conditions, in the absence of antimycin A and at an E_h and 60 mV (curve a) only the second inhibitory effect of FCCP is observed, and the stimulation by PMS and by low concentrations of FCCP is not additive. It seems that stimulation represents maximal turnover of the ATPase. Consequently no E_h optimum is found in the presence of 10^{-6} M FCCP, the ATPase activity remaining high throughout the whole E_h range tested (data not shown).

Figure 7 demonstrates the E_h dependence of ATPase activity, in the absence of P_i, either in the light or in the dark. The optimum is found around 50 mV in the light, and at 75 mV in the dark, which is the same range found for ATPase in the dark, in the presence of P_i. This is also found for higher concentrations of PMS (not shown). Stimulation in the dark by PMS and stimulation in the light (24) might, therefore, proceed via the same pathway, which is also reflected by the fact that both stimulations are sensitive to antimycin A. On the other hand, the stimulation of ATPase by P_i can be

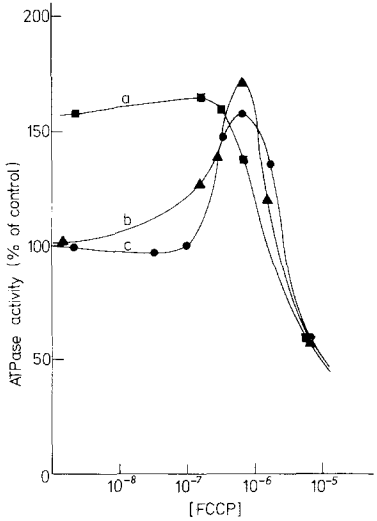

Fig. 6. Dependence on FCCP concentration of ATPase activity, measured in the dark in the presence of P_i. PMS was present at a concentration of 100 μM. Curve a: E_h = 60 mV; curve b: E_h = 60 mV in the presence of antimycin A (1 μg/ml); curve c: E_h = 400 mV. The control rate was 60 μmol per hour per mg BChl.

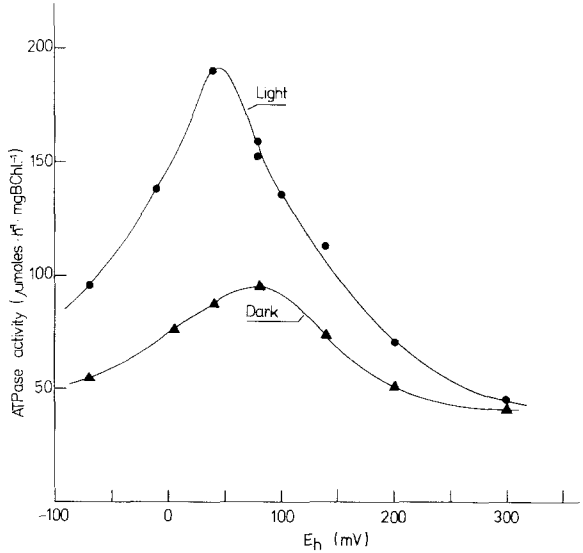

Fig. 7. E_h dependence of ATPase activity in the light and in the dark. ATPase activity was measured as described under Materials and Methods, except that P_i was absent and the concentrations of ATP and Mg^{2+} were 1.5 and 5 mM, respectively. PMS was present at a concentration of 100 μM.

observed at high E_h and undoubtedly represents a different mechanism of activation (compare the rates of ATPase at about 300 mV in Figs. 5 and 7).

Different dependencies of ATPase activity on PMS concentration in light, and in dark in the presence of P_i, are found (Fig. 8). While the former exhibits a normal saturation curve, the latter is inhibited at higher concentrations of PMS. The E_h optimum of either activity is independent of the concentration of PMS as long as stimulation is observed and is always found between 50 and 80 mV. Both activities are only meagerly stimulated by TMPD, and the stimulation is rather independent of TMPD concentration (Fig. 8). Possibly TMPD is just required to poise the endogenous system at optimal E_h, without any additional action. The stimulation of ATPase, in the light, as well as in the dark in the presence of P_i (Fig. 6), is sensitive to antimycin A. In the former case the rate of ATPase decreases from about 190

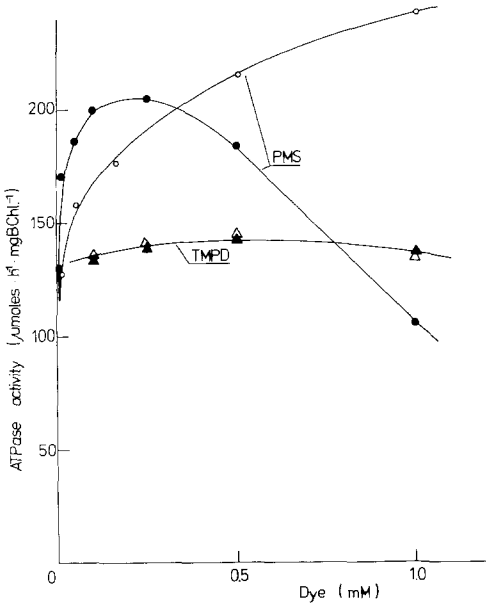

Fig. 8. Dependence of ATPase activity, measured at optimal E_h (80 mV), on the concentration of artifical redox compounds. (O———O) ATPase activity in the presence of PMS, assayed in the light under the conditions described in Fig. 7. (●———●) ATPase activity in the presence of PMS, assayed in the dark under the conditions described in Fig. 6. (▲———▲) ATPase activity in the presence of TMPD, assayed in the dark (Fig. 6). Measurements with TMPD in the light gave essentially identical results (Δ———Δ).

μmol/h^{-1} and mg BChl^{-1} to about 120, in the presence of 1 μg antimycin A per milliliter; in the latter case the rate decreases from about 170 to about 100.

Discussion

The applicability of redox potentiometry for bioenergetic studies has been reviewed by Dutton and Wilson (31). The interpretation of the observed redox dependences is limited by the fact that the PT-electrode can only monitor the redox state of external mediators in some kind of steady-state distribution with the pools of the mediators located within and beyond the membrane. Complete equilibration of all redox components with an external redox mediator would result in complete short-circuiting of the electron transport chain and in the impossibility to perform chemical work. Disequilibrium is therefore a natural requirement for the investigation of an energy transducing system. Nevertheless, our results show that the external redox conditions affect the rates of photophosphorylation and ATPase effectively and reproducibly, indicating that some endogenous component in the cycle is interacting rapidly with external redox compounds. The fact that the E_h optima observed do not depend on the nature of the mediator suggests that the E_h optima are intrinsic properties of endogenous components which are kinetically limiting.

Photophosphorylation

E_h optima between 0 and 100 mV have been reported earlier for cyclic photophosphorylation in chromatophores of photosynthetic bacteria (10–12). In our experiments two sites of interaction for redox mediators with the phosphorylating, cyclic electron transport chain are distinguishable: one is reacting optimally at an external E_h of about 120 mV at pH 8.5, and is observed in the absence of antimycin A at low concentrations of a single redox mediator or of a mixture (Fig. 4). This E_h is between the E_m of a redox carrier Z mediating electron transfer between cytochrome *b* and cytochrome c_2, postulated for *Rps. capsulata* (32) and for *Rps. sphaeroides* (33), and the E_m of cytochrome c_2. In this redox range the rate of electron transfer to cytochrome c_2 is maximal (33, 34), the maximal electric potential across the membrane is formed (35, 36), and also proton uptake following multipulse activation is maximal (37, 38).

A second site of interaction appears at high concentration of PMS or at intermediate concentrations of redox mediators when the redox steady state of the electron transport cycle is perturbed by addition of antimycin A. The

fact that this pathway, in order to be kinetically dominant over the endogenous cycle, or in order to bypass the block by antimycin A, requires relatively high concentrations of mediators suggests that the interaction of this site with the external phase, mediated by the dye, is kinetically more demanding. This second site is characterized by an external E_h of 200 mV at pH 8.5. The reason why other investigators did not find appreciable restoration of photophosphorylation by PMS in chromatophores of *Rps. capsulata* (3, 5) could be due to this narrow optimum at rather high E_h. Restoration has readily been observed in their experiments with *Rhodospirillum rubrum,* and it might be concluded in turn that the two E_h optima lie closer in this case.

It is worth emphasizing that as in *R. rubrum* (6) and in *Rps. capsulata* TMPD, an electron carrier, is as efficient as PMS, a hydrogen carrier, in restoring photophosphorylation; in contrast, for cyclic photophosphorylation in chloroplasts a hydrogen carrier is needed, TMPD being inactive (7, 24) [see the discussion by Trebst (8)]. We therefore propose that TMPD catalyzes a bypass of the antimycin inhibition site, linking the ubiquinone–cytochrome *b* complex with cytochrome c_2 on the inner surface of the chromatophore membrane. The intrinsic proton transport activity of the ubiquinone–cytochrome *b* complex is thereby restored. This would be similar to the bypass of the inhibition site of dibromo-methyl-isoprophyl-*p*-benzoquinone in chloroplasts induced by TMPD in noncyclic (39), as well as in certain types of cyclic photophosphorylation (24). On the basis of the similarities of the reactions, we further suggest that PMS and also DAD restore photophosphorylation in the presence of antimycin A by internal bypasses as TMPD does, and not by transmembrane hydrogen-carrying shuttles, as suggested by Trebst in analogy to the chloroplast system (8). Obviously a fundamental difference in chloroplasts is the very fast reaction of the primary reductant of the photocenter with the endogenous quinone pool in chromatophores (37, 38). Presumably, artificial redox compounds could only compete in this system if the reaction is slowed down, e.g., by extraction of ubiquinone. Preliminary experiments with chromatophores that have been depleted of ubiquinone (40) show that a rather appreciable stimulation of photophosphorylation can be obtained with PMS as mediator. In this case, in analogy to chloroplasts, it is possible that proton translocation is catalyzed by a transmembrane hydrogen-carrying shuttle of PMS. TMPD should be inactive in this case; that remains to be demonstrated (in preparation).

ATPase

ATPase activity of Rhodospirillaceae can be modulated by various chemical and physical parameters, as for example by some anions, divalent

cations, ADP, and light (30, 41). A minimal scheme, which emerges from the studies performed mainly in *Rps. capsulata* (20, 28, 29) and *R. rubrum* (30, 42, 43) chromatophores, must include at least two kinetically distinct forms of the enzyme which have a different activity in energy transduction. An active state of ATPase in *Rps. capsulata* can be achieved either by light via energization of the chromatophore membrane (20) or in the dark by addition of phosphate or other anions (28).

An observed stimulation of ATPase, therefore, can be interpreted in two ways—by a shift of the equilibrium to the more active conformation on one hand, or by a release of the back pressure of the high-energy state of the membrane on the other. Accordingly, the dual effect of the uncoupler FCCP can be explained: at low concentrations it stimulates ATPase by decreasing the high energy state; at higher concentrations the high-energy state becomes too low to maintain the conformational equilibrium of the ATPase in favor of the active form (29) (Fig. 6). We tend to interpret the stimulation of ATPase by PMS, described in this paper, by the second possibility, i.e., as a release of the high-energy pressure via a stimulation of reversed electron flow, as has been suggested for ATPase in mitochondria (13). This is because no further stimulation of ATPase by uncoupler on top of PMS is observed at optimal E_h, but is found at unfavorable E_h (Fig. 6).

The observations for ATPase activated by light resemble those for ATPase activated by P_i (compare Figs. 5 and 7), except that the dependence on PMS concentration is different (Fig. 8): in the light, saturation is observed, while in the dark, inhibition is found at high concentrations of PMS. This can be explained by the assumption that in the light, in spite of fast reversed electron flow, the high-energy state of the membrane is always high enough to keep the ATPase molecules in the more active state, while in the dark, at high PMS concentrations, fast reversed electron flow decreases the high-energy state below the limit necessary for ATPase activation.

The Pathways of Artificial Redox Compounds Catalyzing Photophosphorylation and ATPase

The following considerations have to be included in any electron transport scheme of interpretation.

1. The pathway of PMS and TMPD in photophosphorylating electron flow must be different from that in reversed electron flow, stimulating ATPase. This conclusion is based on the large differences in E_h optima, and on the fact that the former pathway circumvents the antimycin block, while the latter remains largely sensitive to antimycin. Moreover, TMPD is equivalent to PMS in the former, but much less effective in the latter, as

found also for ATPase in mitochondria (13). Of course, since the light reaction in the photocenter is irreversible, for reversed electron flow an additional reaction of the mediator is required for transporting electrons back to cytochrome c_2. Since this would presumably occur across the membrane, the redox couple PMSH/PMS$^{\cdot}$ would be more efficient than reactions involving PMS^+ and $TMPD^+$, because of its higher lipid solubility. In this way the inefficiency of TMPD and the low E_h optimum of the ATPase, which would stabilize the PMSH/PMS$^{\cdot}$ couple, could be explained. However, the antimycin sensitivity of the stimulation of the ATPase tells us, in addition, either that the bypass of the antimycin inhibition site is irreversible, or that in reverse this reaction, together with the transport of reducing equivalents by PMSH back to cytochrome c_2, does not lead to additional dissipation of the high-energy state.

2. The E_h optima for photophosphorylation as well as for ATPase are independent of the E_m of the mediator, and therefore must reflect intrinsic rate limits of the electron transport chain.

3. The rate limit for ATPase apparently does not involve cytochrome c_2, since the same E_h optimum is found for ATPase in the dark and in the light, although cytochrome c_2 is largely reduced under the former but oxidized under the latter condition. This rate limit could involve endogenous ubiquinone, since extraction of this component from chromatophores of *R. rubrum* has been reported to decrease ATPase activity (14).

4. As reflected by the E_h optima, more electrons in the system are needed for the stimulation of ATPase than for endogenous photophosphorylation, which in turn requires more electrons than phosphorylation in the presence of antimycin A.

Simple sequential schemes, as proposed earlier (e.g., in Refs. 5 and 13), without further assumptions are not able to explain why the optimum for the bypass of the antimycin inhibition site is found at 200 mV, which is more oxidizing than the optimum for endogenous cyclic photophosphorylation, and where component Z (32, 33), cytochrome *b*, and PMS should be rather oxidized, even in steady-state electron flow, and why there is no dependence on the E_m of the artificial mediator. They also cannot explain why the bypass in reverse does not lead to an antimycin-insensitive ATPase.

More sophisticated, nonlinear schemes have been proposed for electron transport in mitochondria (44, 45) and in photosynthetic bacteria (15, 16, 44) to explain observations in conflict with a linear scheme. Among them Mitchell's "Q-cycle" is the best-reasoned example (16, 44), and it is possible that our observations could be better explained on this basis also. For instance, it could be assumed that the strict geometry of the cycle is abolished by the action of the artificial redox compound. For this mechanism various

possibilities exist. A particularly interesting one might be in facilitating the dismutation of the semiquinone intermediate formed in the two reaction centers at either surface of the membrane (16, 44). In each case a decrease of the H^+/e^- ratio from 2 to 1 would result, and the transmembrane redox reaction of cytochrome *b* would be lost. It should be tested in this context whether high concentrations of PMS, like antimycin A, inhibit the slow phase of the carotenoid shift (35, 36), as well as the slow binding of the second proton (15, 37).

At the present experimental stage it seems too early for a detailed discussion of our results in view of a nonlinear electron transport scheme. We are inclined to believe, however, that the E_h optimum at 200 mV for photophosphorylation in the presence of antimycin A might again reflect the anomalous behavior of cytochrome *b*, which has lead to the proposal of the "Q-cycle" (16, 44).

Acknowledgments

This work was partially supported by a bilateral research grant from the Consiglio Nazionale delle Ricerche and the Deutsche Forschungsgemeinschaft.

References

1. L. Smith and M. Baltscheffsky, *J. Biol. Chem.* **234** (1959) 1535.
2. D. M. Geller and F. Lipmann, *J. Biol. Chem.* **235** (1960) 2478.
3. J.-H. Klemme and H. G. Schlegel, *Arch. Mikrobiol.* **63** (1968) 154.
4. B. Arwidsson, M. Baltscheffsky, and H. Baltscheffsky, *Biochim. Biophys. Acta* **65** (1962) 429.
5. Z. Gromet-Elhanan and H. Gest, *Archiv. Mikrobiol.* **116** (1978) 29.
6. A. Trebst, E. Pistorius, and H. Baltscheffsky, *Biochim. Biophys. Acta* **143** (1967) 257.
7. A. Trebst, *Ann. Rev. Plant Physiol.* **25** (1974) 423.
8. A. Trebst, *Z. Naturforsch.* **31c** (1976) 152.
9. S. K. Bose and H. Gest, *Proc. Natl. Acad. Sci.* **49** (1963) 337.
10. T. Horio and M. D. Kamen, *Biochemistry* **1** (1962) 144.
11. M. A. Cusanovich, R. G. Bartsch, and M. D. Kamen, *Biochim. Biophys. Acta* **153** (1968) 418.
12. S. A. Culbert-Runquist, R. M. Hadsell, and P. A. Loach, *Biochemistry* **12** (1973) 3508.
13. M. K. F. Wikström and A. M. Lambowitz, *FEBS Lett.* **401** (1974) 49.

14. T. Horio, Y. Horiuti, N. Yamamoto, and K. Nishikawa, in *Methods in Enzymology,* Vol. 24 (A. San Pietro, ed.), Academic Press, New York (1972), pp. 96–103.
15. K. M. Petty, J. B. Jackson, and P. L. Dutton, *FEBS Lett.* **84** (1977) 299.
16. P. Mitchell, G.R.L., 78, 1–6 (1978), grants from Glynn Research Labs. Bodmin, Cornwall, U.K.
17. R. Casadio, A. Baccarini-Melandri, and B. A. Melandri, *FEBS Lett.* **87** (1978) 323.
18. A. Baccarini-Melandri and B. A. Melandri, *Methods in Enzymology,* Vol. 23 (A. San Pietro, ed.), Academic Press, New York (1971), pp. 556–561.
19. M. Avron, *Biochim. Biophys. Acta* **40** (1960) 257.
20. B. A. Melandri, A. Baccarini-Melandri, and E. Fabbri, *Biochim. Biophys. Acta* **275** (1972) 383.
21. T. Horio, K. Nishikawa, and Y. Horiuti, in *Methods in Enzymology*, Vol. 23 (A. San Pietro, ed.), Academic Press, New York (1971), p. 650.
22. R. K. Clayton, *Biochim. Biophys. Acta* **75** (1973) 312.
23. W. M. Clark, *Oxidation Reduction Potentials of Organic Systems,* Williams and Wilkins, Baltimore (1960).
24. G. Hauska, S. Reimer, and A. Trebst, *Biochim. Biophys. Acta* **357** (1974) 1.
25. G. Hauska, W. Oettmeier, S. Reimer, and A. Trebst, *Z. Naturforsch.* **30c** (1975) 37.
26. G. Hauska, A. Trebst, and W. Draber, *Biochim. Biophys. Acta* **305** (1973) 632.
27. A. T. Jagendorf and M. Avron, *J. Biol. Chem.* **231** (1958) 277.
28. A. Baccarini-Melandri, E. Fabbri, and B. A. Melandri, *Biochim. Biophys. Acta* **376** (1975) 82.
29. A. Baccarini-Melandri., E. Fabbri, E. Firstater, and B. A. Melandri, *Biochim. Biophys. Acta* **376** (1975) 72.
30. G. D. Webster and J. B. Jackson, *Biochim. Biophys. Acta,* **503** (1978) 135.
31. P. L. Dutton and D. F. Wilson, *Biochim. Biophys. Acta* **346** (1974) 165.
32. E. H. Evans and A. R. Crofts, *Biochim. Biophys. Acta* **357** (1974) 89.
33. R. C. Prince and P. L. Dutton, *Biochem. Biophys. Acta* **462** (1977) 731.
34. A. R. Crofts and J. Bowyer, in *The Proton and Calcium Pumps* (G. R. Azzone, ed.), Elsevier North-Holland, Amsterdam (1978), pp. 55–62.
35. J. B. Jackson and P. L. Dutton, *Biochem. Biophys. Acta* **325** (1973) 102.
36. A. R. Crofts, D. Browther, and G. V. Tierney, in *Electron Transfer Chains and Phosphorylation* (E. Quagliarello et al., eds.), North-Holland, Amsterdam, (1975), pp. 233–241.
37. R. J. Cogdell, J. B. Jackson, and A. R. Crofts, *J. Bioenergetics* **4** (1973) 211.
38. K. M. Petty and P. L. Dutton, *Arch. Biochem. Biophys.* **172** (1976) 335.
39. A. Trebst and S. Reimer, *Z. Naturforsch* **28c** (1973) 710.
40. A. Baccarini-Melandri and B. A. Melandri, *FEBS Lett.* **80** (1977) 459.
41. B. A. Melandri and A. Baccarini-Melandri, *J. Bioenergetics* **8** (1976) 109.
42. P. A. Edwards and J. B. Jackson, *Eur. J. Biochem.* **62** (1976) 7.
43. G. D. Webster, P. A. Edwards, and J. B. Jackson, *FEBS Lett.* **76** (1977) 29.
44. P. Mitchell, *J. Theor. Biol.* **62** (1976) 327.
45. M. K. F. Wikström and J. A. Berden, *Biochim. Biophys. Acta* **283** (1972) 403.