BBA 46000

UNCOUPLING AND CHARGE TRANSFER IN BACTERIAL CHROMATOPHORES

M. MONTAL*, M. NISHIMURA** AND B. CHANCE***

Department of Biophysics and Physical Biochemistry, Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104 (U.S.A.)

(Received May 12th, 1970)

SUMMARY

Uncoupling of photophosphorylation in bacterial chromatophores is obtained with NH_4Cl or $K^+(Na^+)$ *plus* nigericin in the presence of either valinomycin or suitable permeant anions. The experiments suggest that uncoupling is obtained by the simultaneous abolishment of both pH gradient and membrane potential components of the light-induced electrochemical proton gradient, independently of the nature of the primary event in energy coupling. A general discussion of the relationship between uncoupling and the transfer of charge across the membranes of chromatophores, chloroplasts and submitochondrial particles is presented.

INTRODUCTION

Uncoupling of energy conservation reactions in submitochondrial particles is obtained with a combination of either K^+ and nigericin[1,2] or NH_4Cl[2-5] only in the presence of valinomycin. The effect of valinomycin can be mimicked by appropriate anions, thus suggesting that the role of this ionophore[6] is to allow the electrophoretic efflux of the cationic species (K^+ or NH_4^+) accumulated in the particle, and it may be replaced by an electrophoretic anion influx, obtaining the same uncoupling effect[7,8].

Respiration[4,9,10] in submitochondrial particles or illumination[11-15] of bacterial chromatophores, results in inward translocation of protons, suggesting that the role of the "sidedness" of the system in the coupling event is analogous in both preparations[7,8]. The effects of ionophores on phosphorylation and ion translocation in chromatophores are entirely analogous to those observed in submitochondrial particles[2,4]. Moreover, uncoupling of photophosphorylation in chromatophores occurs in the presence of a combination of K^+ *plus* nigericin and valinomycin[14-16], and inhibition of the light-induced "carotenoid shift" in chromatophores[17,18], a signal that has been associated with the energized state of the system, is observed with NH_4Cl and valinomycin. These analogies between chromatophores and submitochondrial particles

* Present address: Section of Biochemistry and Molecular Biology, Cornell University, Ithaca, N.Y. 14850, U.S.A.

** Permanent address: Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan.

*** To whom reprint requests should be addressed.

prompted us to test whether suitable anions would replace valinomycin in the uncoupling effect observed under the above-mentioned conditions, as occurs in submitochondrial particles[3,4].

MATERIALS AND METHODS

Rhodospirillum rubrum Van Niel Strain 1 was cultured as previously described[19]. After harvesting, cells were broken in a Nossal shaker and chromatophores were isolated with a sucrose–glycylglycine buffer[20,21]. Photophosphorylation was assayed by the glass electrode method of NISHIMURA *et al.*[22]. Bacteriochlorophyll was determined in ether using the absorption coefficient of WEIGL[23]. The antibiotics were generously supplied by Dr. B. C. Pressman.

RESULTS AND DISCUSSION

Table I illustrates the results of the experiments undertaken to prove the above-outlined proposal. HORIO AND YAMASHITA[24] reported that NH_4Cl alone did not uncouple photophosphorylation in chromatophores, thus establishing a difference in the uncoupling process between chloroplasts (where the amines and ammonium are the classical uncouplers[25]) and chromatophores. The fact that in the presence of small amounts of valinomycin, which by itself does not significantly inhibit photophos-

TABLE I

INHIBITION OF PHOTOPHOSPHORYLATION IN CHROMATOPHORES OF *R. rubrum*

Reaction mixture: 20 μmoles NaH_2PO_4, 60 μmoles $MgCl_2$, 2 μmoles sodium succinate, 1 μmole ADP, *R. rubrum* chromatophore bacteriochlorophyll (5.0 μM). Final volume, 4.9 ml; final pH, 7.8; temperature, 25°; excitation light, 850 nm. Pattern of illumination: 20 sec light, 20 sec dark. When indicated, the concentration of valinomycin and nigericin was 0.2 μg/ml. Control rates, 0.6–1.2 ng-ions H^+/bacteriochlorophyll per 20 sec. Assay according to NISHIMURA *et al.*[22].

Addition	*Inhibition* (%)	
	−Valinomycin	*+Valinomycin*
50 μmoles NH_4Cl	—	100
50 μmoles NH_4Cl + 46 nmoles sodium tetraphenylboron	100	100
50 μmoles NH_4Cl + 240 nmoles ammonium picrate	40	100
50 μmoles NH_4Cl + 50 μmoles NaI	4	85
50 μmoles NH_4NO_3	4	88
25 μmoles $(NH_4)_2SO_4$	45	100
50 μmoles ammonium acetate	4	90
46 nmoles sodium tetraphenylboron	45	not tested
50 μmoles KNO_3 (or $NaNO_3$)	—	not tested
50 μmoles KCl (or NaCl)	—	not tested
50 μmoles KNO_3 (or $NaNO_3$) + nigericin	—	not tested
50 μmoles KCl (or NaCl) + nigericin	—	not tested
50 μmoles KNO_3 (or $NaNO_3$) + nigericin + 46 nmoles sodium tetraphenylboron	100	not tested
50 μmoles KCl (or NaCl) + nigericin + 46 nmoles sodium tetraphenylboron	100	not tested
25 μmoles K_2SO_4 (or Na_2SO_4)	20	not tested
25 μmoles K_2SO_4 + nigericin	100	not tested
25 μmoles Na_2SO_4 + nigericin	80	not tested

phorylation[14–16], NH_4Cl completely abolished the phosphorylation reaction, strongly suggests that one of the differences between chloroplasts and chromatophores photophosphorylation resides in the membrane anion selectivity. This is further supported by the fact that micromolar concentrations of tetraphenylboron$^-$ (ref. 26), a lipid-soluble anion that increases several fold the electrical conductance of bilayer lipid membranes[27–30] and proved to be one of the most permeant anions in submitochondrial particles[4, 28], replaces valinomycin in the NH_4^+-dependent uncoupling effect, whereas alone it does not dramatically alter the esterification reaction (Table I) and Fig. 1.

Uncoupling of photophosphorylation in chromatophores is not obtained in the presence of nigericin and K^+, whereas in chloroplasts, nigericin is one of the most powerful uncouplers so far uncovered[31–33]. The presence of valinomycin[14–16] or tetraphenylboron$^-$ results in complete inhibition of photophosphorylation of the nigericin-

TABLE II

UNCOUPLING AND CHARGE TRANSFER IN BIOENERGY-CONSERVING MEMBRANE SYSTEMS

Membrane system	*Abolishment of pH gradient*	*Abolishment of membrane potential*	*Net effect*	*Reference*
Submitochondrial particles	I. $NH_4^+ \xrightarrow{NH_3} H^+$; $H^+ \rightsquigarrow NH_4^+$	Ia. NO_3^- (TPB^-) $\longrightarrow$ in Ib. VAL (NH_4^+) $\longleftarrow$ out	Uncoupling Uncoupling	3, 4 2–5
	II. $K^+ \xrightarrow{N} H^+$; $H^+ \rightsquigarrow K^+$	IIa. NO_3^- (TPB^-) $\longrightarrow$ in IIb. VAL (K^+) $\longleftarrow$ out	Uncoupling Uncoupling	3, 4 2–4
	III. $H^+ \xleftrightarrow{G} H^+$	III. $H^+ \xleftrightarrow{G} H^+$	Uncoupling	40
Chloroplasts	I. $NH_4^+ \xrightarrow{NH_3} H^+$; $H^+ \rightsquigarrow NH_4^+$	I. $Cl^- \longrightarrow$ in	Uncoupling	*cf.* 34
	IIa. $K^+ \xrightarrow{N} H^+$; $H^+ \rightsquigarrow K^+$	II. $Cl^- \longrightarrow$ in	Uncoupling	31–33
	IIb. $K^+ \xleftrightarrow{V} K^+$; $H^+ \xleftrightarrow{F} H^+$	II. $Cl^- \longrightarrow$ in	Uncoupling	38
	III. $H^+ \xleftrightarrow{G} H^+$	III. $H^+ \xleftrightarrow{G} H^+$	Uncoupling	32, 37, 38
Chromatophores	I. $NH_4^+ \xrightarrow{NH_3} H^+$; $H^+ \rightsquigarrow NH_4^+$	Ia. TPB^- (SO_4^{2-}) $\longrightarrow$ in Ib. VAL (NH_4^+) $\longleftarrow$ out	Uncoupling Uncoupling	This work This work, 17
	II. $K^+ \xrightarrow{N} H^+$; $H^+ \rightsquigarrow K^+$	IIa. TPB^- (SO_4^{2-}) $\longrightarrow$ in IIb. VAL (K^+) $\longleftarrow$ out	Uncoupling Uncoupling	This work 14–16
	III. $H^+ \xleftrightarrow{G} H^+$	III. $H^+ \xleftrightarrow{G} H^+$	Uncoupling	14

Abbreviations: VAL, (V) valinomycin; N, nigericin; F, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; G, gramicidin; TPB^-, tetraphenylboron$^-$.

treated chromatophore, in complete agreement with the results obtained in submitochondrial particles[3,4] (Table II). As illustrated in Fig. 1, lower concentrations of tetraphenylboron$^-$ were required to completely inhibit photophosphorylation when nigericin was present. It must be emphasized, however, that the nonphosphorylating light-induced pH rise in NaCl was considerably enhanced by tetraphenylboron$^-$ in both *Chromatium* and *R. rubrum* chromatophores (Fig. 2). Picrate[28,29] and SO_4^{2-} were also active in the substitution of valinomycin for the NH_4^+ and K^+ *plus* nigericin-dependent inhibition of photophosphorylation, although not to the same extent as tetraphenylboron$^-$.

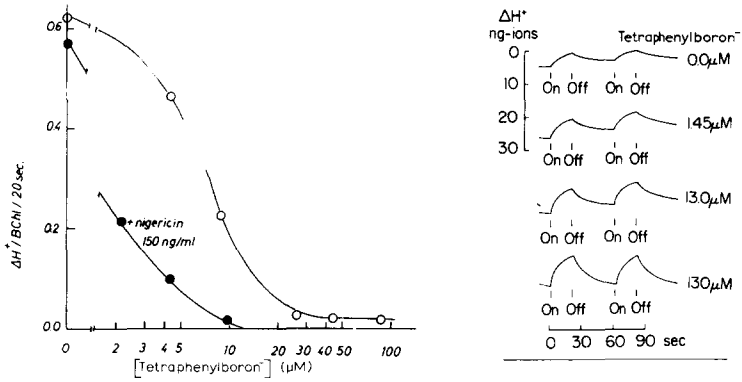

Fig. 1. Effects of tetraphenylboron$^-$ and nigericin on photophosphorylation of *Chromatium* chromatophores. Chromatophores, 69.6 nmoles bacteriochlorophyll/4.6 ml; phosphorylating medium at pH 7.8 (no K^+). Illumination, tungsten lamp + Wratten 88-A filter (>720 nm) + water layer (4.5 cm thickness), 42 kergs/cm^2·sec. Temperature, 24°. Linear portion of the pH rise was used for calculation.

Fig. 2. Effect of tetraphenylboron$^-$ on the light-induced pH rise of *R. rubrum* chromatophores in NaCl. Chromatophores, 62.2 nmoles bacteriochlorophyll/4.6 ml; 50 mM NaCl of pH 6.5. Illumination, tungsten lamp + Wratten 88-A filter (>720 nm) + water layer (4.5 cm thickness), 42 kergs/cm^2·sec. Temperature, 23°.

Therefore, uncoupling in chromatophores is associated with the abolishment of the light-induced pH gradient[7,8] either by the electrically neutral K^+ (or Na^+)/H^+ exchange catalyzed by nigericin[6,14–16] or by the internal protonation of the freely diffusible NH_3 (*cf.* ref. 34), and the collapse of the light-induced membrane potential[7,8] either by the electrophoretic cation efflux mediated *via* valinomycin[6,14–16] or by the electrophoretic anion influx observed with highly lipid-soluble anions. This last point is further substantiated by the fact that both valinomycin[14–16] and tetraphenylboron$^-$ (Fig. 2) stimulate both the rate and extent of the light-induced pH rise, thus suggesting that the rate-limiting step is the transfer of charge associated with H^+ translocation, which is neutralized by the electrophoretic ion migration.

It has been inferred[34,35] that the chloroplast membrane is permeable to Cl^-; a direct demonstration of this suggestion has been provided recently by DEAMER AND PACKER[36] who showed that illumination of chloroplasts suspended in a solution of $Na^{36}Cl$ results in the stoichiometric uptake of H^+ and Cl^-. Thus, one would expect that the dominant component of the electrochemical proton gradient created by the photoevent would be the pH gradient[7,8,34]. In this case, valinomycin would not and indeed does not[37,38] uncouple photophosphorylation in chloroplasts; however, it is

worth noting that the rate of the light-induced pH rise is markedly enhanced by prior addition of valinomycin to the incubation medium[38], thus suggesting that the rate-limiting step is not the translocation of H^+ itself but the associated transfer of charge[7,8] which is dissipated by allowing the electrophoretic efflux of K^+ *via* valinomycin. Uncoupling in chloroplasts is obtained with KCl and nigericin[31–33] in submitochondrial particles with KNO_3 or KCl *plus* tetraphenylboron$^-$ and nigericin[3,4] and in chromatophores with K_2SO_4 (or Na_2SO_4) or KCl (or NaCl) *plus* tetraphenylboron$^-$ and nigericin. Uncoupling is obtained with NH_4Cl in chloroplasts[25,34,39], with NH_4NO_3 (or NH_4Cl *plus* tetraphenylboron$^-$) in submitochondrial particles[3,4], and with $(NH_4)_2SO_4$ (or NH_4Cl *plus* tetraphenylboron$^-$) in chromatophores. These results strongly suggest that the intimate mechanism of uncoupling of energy conservation reactions is the same for submitochondrial particles, chromatophores and chloroplasts, but some of the differences are due to membrane anion selectivity, the permeant anion in submitochondrial particles being NO_3^{2-} (ref. 2), tetraphenylboron$^-$ (refs. 3, 4, 28) or picrate (refs. 3, 4, 28), in chromatophores SO_4^{2-}, tetraphenylboron$^-$ or picrate, and in chloroplasts Cl^- (ref. 36). It is of interest to point out that subchloroplast particles obtained by sonic disruption behave analogously to submitochondrial particles and chromatophores. In other words, they are uncoupled by a combination of NH_4Cl or KCl *plus* nigericin and valinomycin and it is possible that the membrane anion selectivity may be altered by sonication[39].

Table II presents a summary of the conditions of uncoupling and charge transfer in submitochondrial particles, chromatophores and chloroplasts, energy-conserving membrane systems with the same sidedness of proton translocation and location of coupling factor 1 in direct contact with the environmental solution[7,8,41–44], specifically pointing out the mechanisms by which the components of the energized state of the system are affected and the net result obtained. As was proposed[7,8] the energized state of these organelles is associated with both a pH gradient and a membrane potential, and uncoupling is obtained only when both components of the respiration (submitochondrial particles) or light (chromatophores and chloroplasts)-induced electrochemical proton gradient are abolished. It is still an open question what is the primary event in the energy-coupling process; whether the anisotropic distribution of electron and H-atom carriers in the coupling membrane is nature's device to provide a source of electrical potential to energy-conserving membranes[7,8] or whether primary electron-transfer linked conformational alterations induce secondary charge separation in or across the membrane, or alter the proton-binding affinity of membrane groups (the "membrane Bohr effect")[45,46] cannot be decided by our experiments.

ACKNOWLEDGMENTS

The authors are indebted to Drs. A. R. Crofts, and R. E. McCarty for many valuable discussions. This work has been supported by grants from the National Institute of Health GM-12202 and the National Institute for Scientific Research (Mexico).

REFERENCES

1 M. Montal, B. Chance, C. P. Lee and A. Azzi, *Biochem. Biophys. Res. Commun.*, 34 (1969) 104.
2 R. S. Cockrell and E. Racker, *Biochem. Biophys. Res. Commun.*, 35 (1969) 414.
3 M. Montal, B. Chance and C. P. Lee, *Biochem. Biophys. Res. Commun.*, 36 (1969) 428.

4 M. MONTAL, B. CHANCE AND C. P. LEE, *J. Membrane Biol.*, 2 (1970) 201.
5 S. PAPA, in S. PAPA, J. M. TAGER, E. QUAGLIARIELLO AND E. C. SLATER, *The Energy Level and Metabolic Control in Mitochondria*, Adriatica Editrice, Bari, 1969, p. 273.
6 B. C. PRESSMAN, E. J. HARRIS, W. S. JAGGER AND J. H. JOHNSON, *Proc. Natl. Acad. Sci. U.S.*, 58 (1967) 1949.
7 P. MITCHELL, *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glynn Research, Bodmin, Cornwall, 1966.
8 P. MITCHELL, *Chemiosmotic Coupling and Energy Transduction*, Glynn Research, Bodmin, Cornwall, 1968.
9 P. MITCHELL AND J. MOYLE, *Nature*, 208 (1965) 1205.
10 B. CHANCE AND L. MELA, *J. Biol. Chem.*, 242 (1967) 830.
11 B. CHANCE, M. NISHIMURA, M. AVRON AND M. BALTSCHEFFSKY, *Arch. Biochem. Biophys.*, 117 (1966) 158.
12 L. V. VON-STEDINGK AND H. BALTSCHEFFSKY, *Arch. Biochem. Biophys.*, 117 (1966) 400.
13 M. NISHIMURA, K. KADOTA AND B. CHANCE, *Arch. Biochem. Biophys.*, 125 (1968) 308.
14 J. B. JACKSON, A. R. CROFTS AND L. V. VON-STEDINGK *European J. Biochem.*, 6 (1968) 41.
15 M. NISHIMURA AND B. C. PRESSMAN, *Biochemistry*, 8 (1969) 1360.
16 A. D. THORE, D. L. KEISTER, N. SHAVIT AND A. SAN PIETRO, *Biochemistry*, 7 (1968) 3499.
17 D. E. FLEISHMANN AND R. K. CLAYTON, *Photochem. Photobiol.*, 8 (1968) 287.
18 M. NISHIMURA, *Biochim. Biophys. Acta*, 197 (1970) 69.
19 M. NISHIMURA AND B. CHANCE, *Biochim. Biophys. Acta*, 66 (1963) 17.
20 M. NISHIMURA, *Biochim. Biophys. Acta*, 57 (1962) 88.
21 M. NISHIMURA, *Biochim. Biophys. Acta*, 57 (1962) 96.
22 M. NISHIMURA, T. ITO AND B. CHANCE, *Biochim. Biophys. Acta*, 59 (1962) 177.
23 J. W. WEIGL, *J. Am. Chem. Soc.*, 75 (1953) 999.
24 T. HORIO AND J. YAMASHITA, *Biochim. Biophys. Acta*, 88 (1964) 237.
25 D. W. KROGMAN, A. T. JAGENDORF AND M. AVRON, *Plant Physiol.*, 34 (1959) 277.
26 K. UTSUMI AND L. PACKER, *Arch. Biochem. Biophys.*, 122 (1967) 509.
27 P. MUELLER AND D. O. RUDIN, *Biochem. Biophys. Res. Commun.*, 26 (1967) 398.
28 E. A. LIBERMAN, V. P. TOPALY, L. M. TSOFINA, A. A. JASAITIS AND V. P. SKULACHEV, *Nature*, 222 (1969) 1076.
29 E. A. LIBERMAN AND V. P. TOPALY, *Biochim. Biophys. Acta*, 163 (1968) 125.
30 O. H. LEBLANC, JR., *Biochim. Biophys. Acta*, 193 (1969) 350.
31 N. SHAVIT AND A. SAN PIETRO, *Biochem. Biophys. Res. Commun.*, 28 (1967) 277.
32 N. SHAVIT, R. A. DILLEY AND A. SAN PIETRO, *Biochemistry*, 7 (1968) 2356.
33 L. PACKER, *Biochem. Biophys. Res. Commun.*, 28 (1967) 1022.
34 A. R. CROFTS, *J. Biol. Chem.*, 242 (1967) 3352.
35 G. HIND, H. Y. NAKATANI AND S. IZAWA, *Biochim. Biophys. Acta*, 172 (1969) 277.
36 D. W. DEAMER AND L. PACKER, *Biochim. Biophys. Acta*, 172 (1969) 539.
37 M. AVRON AND N. SHAVIT, *Biochim. Biophys. Acta*, 109 (1965) 317.
38 S. J. D. KARLISH, N. SHAVIT AND M. AVRON, *European J. Biochem.*, 9 (1969) 291.
39 R. E. MCCARTY, *J. Biol. Chem.*, 244 (1969) 4292.
40 M. MONTAL, B. CHANCE AND C. P. LEE, *FEBS Letters*, 6 (1970) 209.
41 V. K. VAMBUTAS AND E. RACKER, *J. Biol. Chem.*, 240 (1965) 2660.
42 D. F. PARSONS, W. D. BONNER AND J. G. VERBOON, *Can. J. Botany*, 43 (1965) 647.
43 S. H. HOWELL AND E. N. MOUDRIANAKIS, *Proc. Natl. Acad. Sci. U.S.*, 58 (1967) 261.
44 H. LÖW AND B. A. AFZELIUS, *Exptl. Cell Res.*, 35 (1964) 431.
45 B. CHANCE, G. K. RADDA AND C. P. LEE, in J. M. TAGER, S. PAPA, E. QUAGLIARIELLO AND E. C. SLATER, *Electron Transfer and Energy Conservation*, Adriatica Editrice, Bari, in the press.
46 B. CHANCE, A. R. CROFTS, M. NISHIMURA AND B. PRICE, *European J. Biochem.*, 13 (1970) 364.