Biochem. J. (1982) **206**, 351–357
Printed in Great Britain

On the extent of localization of the energized membrane state in chromatophores from *Rhodopseudomonas capsulata* N22

G. Duncan HITCHENS and Douglas B. KELL*
Department of Botany and Microbiology, University College of Wales, Aberystwyth, Dyfed SY23 3DA, Wales, U.K.

(*Received 15 March 1982/Accepted 19 May 1982*)

1. The principle of the double-inhibitor titration method for assessing competing models of electron transport phosphorylation is expounded. 2. This principle is applied to photophosphorylation by chromatophores from *Rhodopseudomonas capsulata* N22. 3. It is found that, in contrast to the predictions of the chemiosmotic coupling model, free energy transfer is confined to individual electron transport chain and ATP synthase complexes. 4. This conclusion is not weakened by arguments concerning the degree of uncoupling in the native chromatophore preparation or the relative number of electron transport chain and ATP synthase complexes present. 5. Photophosphorylation is completely inhibited by the uncoupler SF 6847 at a concentration corresponding to 0.31 molecules per electron transport chain. 6. The apparent paradox is solved by the proposal, consistent with the available evidence on the mode of action of uncouplers, that uncoupler binding causes a co-operative conformational transition in the chromatophore membrane, which leads to uncoupling and which is not present in the absence of uncoupler.

It is now widely accepted that bacterial chromatophores provide an excellent system for studies aimed at furthering our understanding of the processes involved in electron transport phosphorylation (Crofts & Jackson, 1970; Crofts, 1974; Gromet-Elhanan, 1977; Crofts & Wood, 1978; Clayton & Sistrom, 1978; Baccarini-Melandri *et al.*, 1981). In particular, they provide an excellent experimental opportunity to attack the important current problem of whether the energized intermediate between electron transport and ADP phosphorylation is delocalized over the entire chromatophore membrane, as in the chemiosmotic model of membrane energy coupling processes (e.g. Mitchell, 1966, 1979*a*,*b*), or whether there exist more localized and direct free-energy-transferring interactions between electron transport complexes and ATP synthase complexes in these energy-coupling membranes (e.g. Del Valle-Tascon *et al.*, 1978; Williams, 1978; Kell, 1979; Petty & Jackson, 1979*b*; Melandri *et al.*, 1980, 1981; Baccarini-Melandri *et al.*, 1981; Conover & Azzone, 1981).

Since a great deal of uncertainty remains concerning the quantitative reliability of methods for measuring the bulk-phase protonmotive force in bacterial chromatophores (e.g. Elema *et al.*, 1978; Kell *et al.*, 1978; Michels & Konings, 1978; Ferguson *et al.*, 1979; Kell, 1979; Ostroumov *et al.*, 1979; Armitage & Evans, 1981; Baccarini-Melandri *et al.*, 1981; Casadio *et al.*, 1981; Cirillo & Gromet-Elhanan, 1981; Clark & Jackson, 1981; Jackson & Clark, 1981), we have chosen to examine the functional interactions between electron transport and ATP synthase complexes by means of the use of double-inhibitor titrations. The principle of this method (Fig. 1) (cf., e.g., Baum *et al.*, 1971; Kell *et al.*, 1979; Melandri *et al.*, 1981) is that, in the case of electron transport phosphorylation, a titration curve of the rate of phosphorylation versus the concentration of a specific electron transport inhibitor may be established. Then an aliquot of the energy-coupling-membrane preparation may be taken and reacted with a concentration of a covalent ATPase inhibitor sufficient to reduce the rate of phosphorylation, in the absence of added electron transport inhibitor, by, say, 50% compared with that of the control membranes. Under these conditions, the rate-limiting reaction for the phosphorylation is evidently associated with the turnover of the ATPase enzymes themselves, and no strong rate limitation is associated either with the turnover of individual

Abbreviations used: SF 6847, 3,5-di-*t*-butyl-4-hydroxybenzylidene malononitrile; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

* To whom correspondence should be addressed.

0306-3283/82/080351-07$01.50/1

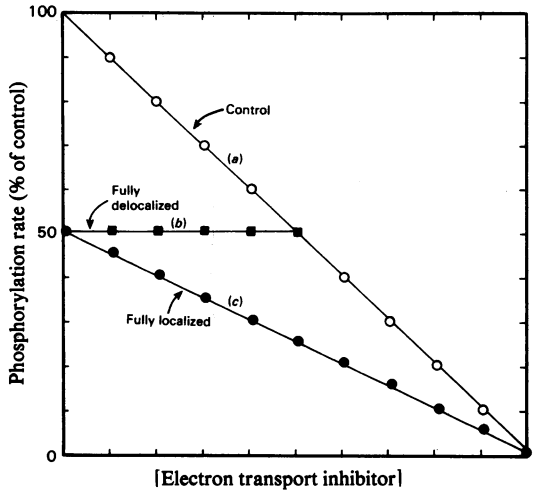

Fig. 1. *Principle of the double-inhibitor titration method for assessing the extent of localization of free energy transfer in bioenergetic systems*

(*a*) A titration curve is established of the effect of an electron transport inhibitor on phosphorylation by the membrane vesicle preparation under study (○). Then the membranes are reacted with a covalent ATP synthase inhibitor such that their phosphorylation rate is decreased to, say, 50% of the control rate (■, ●). The titration is repeated, using the same electron transport inhibitor, and two idealized limiting cases may be anticipated. In (*b*), the free energy transfer is fully delocalized amongst all electron transport and ATPase complexes on a given membrane vesicle, as in the chemiosmotic model. In (*c*), in a model of the opposite extreme, in which free energy transfer is restricted to particular electron transport and ATPase complexes, although the rate-limitation was notionally originally at the level of the ATPases, the electron transport inhibitor is just as effective as in the control titration. For further discussion, see the text.

electron transport chains or the provision of energy to the functioning ATPases. If the effect of the same electron transport inhibitor upon phosphorylation by the latter preparation, some, say one-half, of whose ATPases have been inhibited with the covalent ATPase inhibitor, is tested, two possible limiting titration curves (Fig. 1) may be observed. If the intermediate generated by electron transport is fully delocalized amongst all ATPases in a given chromatophore, no initial effect of the electron transport inhibitor upon the observed rates of phosphorylation will be expected (Fig. 1*b*), since the rate of phosphorylation is supposedly limited by the turnover of the ATPase enzymes, In contrast, for a model of the opposite extreme, in which the energized intermediate generated by a particular electron transport chain may be utilized only by a particular ATPase, the electron transport inhibitor will be just as effective as in the control experiment (Fig. 1*c*). Thus a simple distinction is possible between 'localized' and 'delocalized' models of electron transport phosphorylation in chromatophores.

The purpose of the present article is to report an experimental analysis of electron transport phosphorylation in bacterial chromatophores by using the double-inhibitor titration technique described above. It is found, using this technique, that the localization of free-energy transfer during electron transport phosphorylation by bacterial chromatophores is apparently complete. However, it is also found that full uncoupling of electron transport from phosphorylation may be observed with the uncoupler SF 6847 at a concentration of uncoupler equivalent to 0.31 molecules per electron transport chain. The apparent paradox between this result and that from the double-inhibitor titrations is simply resolved by proposing that the presence of the uncoupler itself can sub-stoichiometrically cause a co-operative uncoupling transition in the energy-coupling proteins of the chromatophore membrane.

Experimental

Biological material

Rhodopseudomonas capsulata N22, generously given by Dr. J. B. Jackson, was grown phototrophically under anaerobic conditions at 30 ± 1°C in filled 500 ml flasks, in a medium containing, per litre: $(NH_4)_2SO_4$, 1 g; sodium DL-malate, 4 g; KH_2PO_4, 0.6 g; K_2HPO_4, 0.48 g; sodium EDTA, 20 mg; $MgSO_4,7H_2O$, 0.2 g; $CaCl_2$, 75 mg; $FeSO_4,7H_2O$, 12 mg; thiamine HCl, 1 mg; $MnSO_4$, 4 mg; H_3BO_3, 7 mg; $Cu(NO_3)_2,8H_2O$, 0.1 mg; $ZnSO_4,7H_2O$, 0.6 mg; $NaMoO_4,2H_2O$, 1.9 mg; pH 6.8. For the preparation of chromatophores, all procedures were carried out at 4°C. Mid-exponential phase cultures were washed once and resuspended to a volume of 50 ml in a medium containing 50 mM-Tricine/NaOH/50 mM-NaCl/8 mM-$MgCl_2$, pH 7.4 (Packham *et al.*, 1978). Chromatophores were prepared by sonication (3 × 30 s) at the full power of an MSE 150 watt ultrasonic disintegrator, operating at 20 μm peak-to-peak amplitude. Cell debris was removed by centrifugation at 20000 *g* for 15 min, and chromatophores were sedimented at 115000 *g* for 90 min. The chromatophores were resuspended by using a glass homogenizer in approx. 10 ml of a medium containing 3 mM-KH_2PO_4/10 mM magnesium acetate/30 mM-potassium acetate, pH 7.8, and were stored in the dark at 0–4°C until required. Bacteriochlorophyll concentrations were estimated after extraction with acetone/methanol (7:2, v/v) using the extinction coefficients given by Clayton (1963). Bacteriochlorophyll concentrations in typical stock

chromatophore preparations were in the range 0.2–0.4 mM.

Photophosphorylation

Photosynthetic phosphorylation was measured by a modification of the method of Nishimura *et al.* (1962), in a black-painted glass reaction vessel maintained at 25°C by a thermostat. Illumination was effected by means previously described (Ferguson *et al.*, 1979), and control experiments in which the chromatophore concentration was varied were performed to establish that the light intensity under these conditions was saturating for photophosphorylation. The reaction medium for photophosphorylation assays contained, in a final volume of 6 ml, 3 mM-KH_2PO_4, 10 mM-magnesium acetate, 30 mM-potassium acetate, 0.2 mM-sodium succinate, 1.5 mM-sodium ADP, pH 7.8, chromatophores corresponding to a bacteriochlorophyll concentration of 20 μM and 800 μg of carbonic anhydrase. In addition, P^1,P^5-bis-(5′-adenosyl)pentaphosphate an inhibitor of adenylate kinase (Lienhard & Secemski, 1973), was included at a final concentration of 5 μM, since its presence caused a slight stimulation of the net rate of photophosphorylation. Reaction mixtures were vigorously stirred with a magnetic follower and were maintained under a stream of CO_2-free oxygen. No effect of adenosine 5′-[β,γ-imido]triphosphate (0.2 mM) upon the rate of phosphorylation was observed, indicating that negligible uncoupled ATP hydrolase activity was present in these preparations. The scalar photophosphorylation reaction is accompanied, at pH 7.8, by the uptake of approximately 0.95 H^+ (Nishimura *et al.*, 1962), and the decrease in pH caused by this reaction was used to determine the rate of photophosphorylation, measured by using a Russell pH electrode connected to an Orion 701 Ionalyser, whose output was fed to a Linseis LS 64 potentiometric chart recorder. Changes in pH were converted to rates of ATP synthesis by using calibrated additions of HCl and KOH, and the conversion coefficients given by Nishimura *et al.* (1962).

Chemicals

Antimycin A, P^1,P^5-bis-(5′-adenosyl)pentaphosphate, adenosine 5′-[β,γ-imido]triphosphate and dicyclohexylcarbodi-imide were purchased from Sigma. Compound SF 6847 was generously given by Dr. Y. Nishizawa, Sumitomo Chemical Company, Osaka, Japan. Other reagents were obtained from Sigma or BDH, and were of the highest grade available. Water was doubly distilled in an all-glass apparatus.

Results

Fig. 2 shows a trace of the scalar light-induced pH increase accompanying the phosphorylation of ADP,

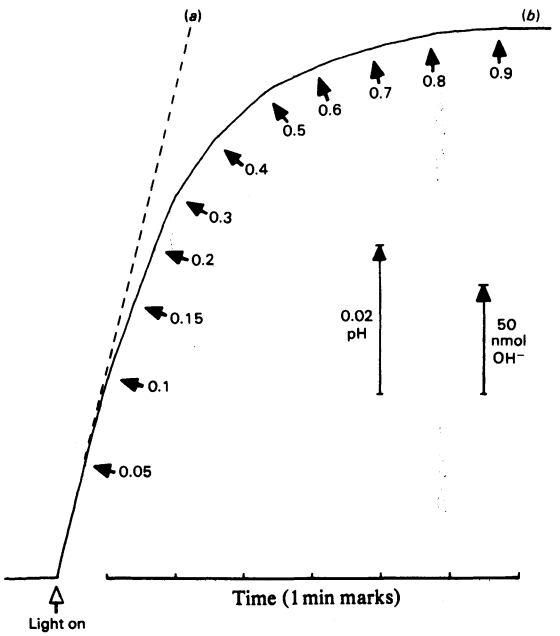

Fig. 2. *Photophosphorylation in Rps. capsulata chromatophores, and its inhibition by antimycin A*
Photophosphorylation was carried out as described in the Experimental section. At the point indicated, illumination was effected, and a black cloth was removed from over the reaction vesssl. In trace (*a*) (---), no further additions were made, whilst in trace (*b*) (——), antimycin A was added at the points, and to the micromolar concentrations, indicated.

measured as described in the Experimental section. Fig. 2(*a*) shows that this rate is constant, despite the build-up of ATP, throughout the time, phosphorylation potential and pH change considered, whilst Fig. 2(*b*) shows that the rate of phosphorylation is decreased by the addition of the electron transport inhibitor antimycin A. Similar data for *Rps. sphaeroides* chromatophores, together with strong evidence that this inhibition by antimycin A is not due to any uncoupling (cf. Wikström & Krab, 1979), have been provided by Melandri *et al.* (1981). A separate aliquot of chromatophores from the same preparation was preincubated with dicyclohexylcarbodi-imide (50 μM) in the reaction vessel for 25 min in the dark, and a similar titration of photophosphorylation to that of the control chromatophores shown in Fig. 2(*b*) was performed with antimycin A. The results of the two titrations are shown in Fig. 3. It is clear that the relative titration curves correspond quite closely to the idealized plots given in Fig. 1(*c*), indicating that the energy coupling process between individual electron transport chains and ATPase enzymes is completely

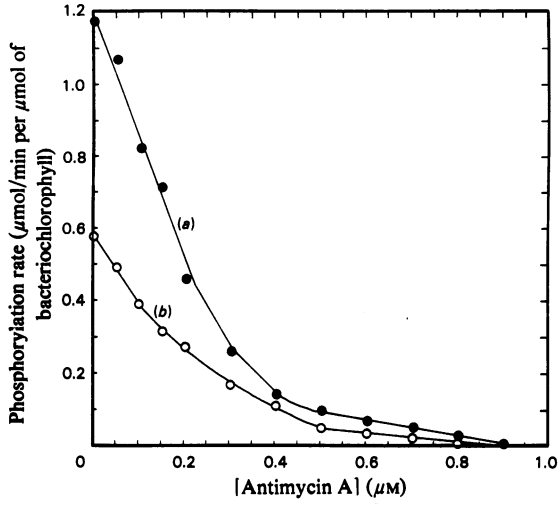

Fig. 3. *Effect of dicyclohexylcarbodi-imide and antimycin A on phosphorylation by Rps. capsulata chromatophores*

The rate of photophosphorylation, performed as described in the Experimental section, is plotted against the concentration of the electron transport inhibitor antimycin A for two cases. In trace (*a*), the data from Fig. 2 (*b*) are plotted. An aliquot of chromatophores from the same preparation was reacted with 50 μM-dicyclohexylcarbodi-imide as described in the text, and the titration was repeated. It may be observed that these titration curves correspond rather closely to the idealized titration curves for fully localized energy transfer given in Figs. 1(*a*) and 1(*c*).

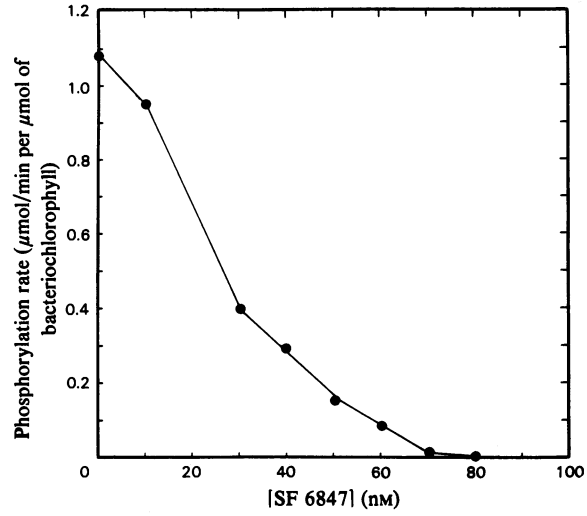

Fig. 4. *Effect of uncoupler SF 6847 on photophosphorylation by Rps. capsulata chromatophores*

Photophosphorylation was measured as described in the legend to Fig. 2, and small volumes of SF 6847 in ethanol were added to the concentrations indicated.

localized, without even a hint of delocalization, Similar data (not shown) were obtained when the amount of dicylohexylcarbodi-imide added was varied so as to inhibit phosphorylation by between 30 and 70% of the rate observed in its absence. A similar conclusion concerning the lack of energetic interaction between the different electron transport and ATPase complexes, together with evidence presented to show that, as expected, dicyclohexylcarbodi-imide does not act as an electron transport inhibitor, was reached by Melandri *et al.* (1981) on the basis of double-inhibitor titrations under single-turnover-flash conditions (and cf. Baccarini-Melandri *et al.*, 1977; Casadio *et al.*, 1978).

However, earlier work with chromatophores from *Rps. sphaeroides* using the ionophore valinomycin (Saphon *et al.*, 1975), and, in particular, experiments with rat-liver mitochondria using the uncoupler SF 6847 (Terada & van Dam, 1975) have shown that uncoupling can occur at concentrations of uncoupler of less than 1 molecule per electron transport chain. We have therefore carried out a titration of the rate of photophosphorylation by *Rps. capsulata* chromatophores with the uncoupler SF 6847, with the resulting data plotted in Fig. 4. It is seen that full uncoupling of photophosphorylation is caused by 80 nM-SF 6847 when chromatophores corresponding to 20 μM-bulk bacteriochlorophyll are present. Since there is 1 reaction centre for approx. every 77 molecules of bulk bacteriochlorophyll (Packham *et al.*, 1978), the maximum number of functioning electron transport chains under the conditions of Fig. 4 is equivalent to 260 nM. Thus, even assuming that every added molecule of SF 6847 is active, full uncoupling is achieved by 0.31 molecules of SF 6847 per electron transport chain. Thus although the energy-coupling process appears to be completely localized, uncoupling seems to exhibit co-operative or delocalized features.

Discussion

As indicated in Fig. 1, the use of double-inhibitor titrations offers, in principle, a simple and clear-cut tool for making an experimental distinction between competing models of electron transport phosphorylation. The data in Fig. 3 indicate that in bacterial chromatophores, and in contrast to the predictions of the chemiosmotic coupling hypothesis, the energetic coupling between the reactions of the electron transport chains and the ATP synthases is quite localized in nature, and does not extend over the entire chromatophore membrane vesicle. We now consider alternative interpre-

tations of these experiments that might make such a conclusion unwarranted.

One possibility, discussed by Baum *et al.* (1971), is that there is a significant native 'energy leak' in the system. Thus, if electron transport-derived free energy is partitioned between ATP synthesis and a significant competing energy 'leak', inhibition of either electron transport or the ATP synthase enzymes would, even in the chemiosmotic coupling model, lead to a decrease in photophosphorylation, since a greater proportion of the electron transport-derived energy would go to the energy 'leak'. Amongst the abundant evidence that bacterial chromatophores do not in general have a significant energy leak of this type are the following: (i) the phosphorylation rates of our chromatophores ($>1\,\mu$mol of ATP$\cdot$min$^{-1}\cdot\mu$mol of bacteriochlorophyll^{-1}) compare favourably with those obtained by others (e.g. Petty & Jackson, 1979*a*); (ii) under multiple-flash conditions the P/2e$^-$ ratio of bacterial chromatophores is close to the maximal theoretical efficiency of a system with the calculated degree of coupling (Jackson *et al.*, 1981) and, more importantly, is essentially independent of the flash frequency when this is varied between 3.3 and 12.5 Hz (Melandri *et al.*, 1981); (iii) no rapid decay phase of the electrochromic carotenoid response ascribable to significant uncoupling is observed in bacterial chromatophores (Jackson *et al.*, 1978). We therefore conclude that the present results are not attributable to the presence in chromatophores of a significant energy 'leak'.

Another situation (J. B. Jackson, personal communication) that might artefactually lead to the conclusion that the energy coupling process was localized, rather than chemiosmotic, in nature, would arise if bacterial chromatophore preparations contained a significant fraction of chromatophore vesicles that possessed but one or two ATPase molecules. The evidence that this is not the case includes the following: (i) titrations of *Rps. capsulata* chromatophores with the tight-binding and specific ATPase inhibitors venturicidin, efrapeptin and 4-chloro-7-nitrobenzofurazan all suggest that there is at least 1 ATPase per 100–200 bulk bacteriochlorophyll molecules (Petty & Jackson, 1979*a*), i.e. >5–10 ATPases per chromatophore; if the ATPases per chromatophore were distributed normally, an insignificant fraction of chromatophores would have only 1 or 2 ATPases; (ii) electron microscopic data demonstrates rather clearly that bacterial chromatophores contain ATPases and reaction centre complexes in approximately equal amounts (Reed & Raveed, 1972); (iii) the presence of any chromatophores containing only 1 or 2 ATPases would have caused an initial shoulder in the antimycin A titration curves (Fig. 3), which was not observed. We thus retain the conclusion that, in contrast to the requirements of the chemiosmotic coupling model, free energy transfer during photophosphorylation by bacterial chromatophores is not delocalized over the entire chromatophore vesicle, but appears, in the absence of protonophores or ionophores, to be localized between individual electron transport chain and ATPase complexes.

However, it was found (Fig. 4) that the potent uncoupler SF 6847 caused complete uncoupling of electron transport phosphorylation at a concentration of 0.31, i.e. <1, molecule per electron transport chain. In fact this is a minimal estimate of its potency, since the actual concentration of active SF 6847 molecules bound to the chromatophore membranes is unknown. In discussing the potency of SF 6847 in uncoupling rat liver mitochondria, in which complete uncoupling occurred at an SF 6847 concentration of 0.2 molecules per respiratory chain (0.06 molecules per 'coupling site'), Terada & van Dam (1975) considered two possibilities. The first was that the uncoupler molecules themselves could move rapidly between sites, with, in this case, only 6% of coupling sites active at any one moment (Margolis *et al.*, 1967). The second possibility was that all coupling sites in a given vesicle released their free energy to a common pool, as in the chemiosmotic coupling theory, and the uncoupler molecules worked at full speed (perhaps as protonophores) to dissipate this supply. Although there is abundant evidence that a variety of uncouplers can act in this way to shuttle H^+ across the bilayer portions of phospholipid membranes (McLaughlin & Dilger, 1980), such a view would be inconsistent with the data from the double inhibitor titrations (Fig. 3) discussed earlier. Further, the latter model would not be consistent with the following, more recently discovered, features of uncoupling (Kell, 1981): (i) the presence in mitochondria, at a concentration of approx. 1 per respiratory chain, of high-affinity proteinaceous uncoupler-binding sites (Hatefi, 1975; Hanstein, 1976*a*,*b*; Katre & Wilson, 1978); (ii) the existence of a number of uncoupler-resistant mutant strains of bacteria (Decker & Lang, 1977, 1978; Guffanti *et al.*, 1981; Ito & Ohnishi, 1981) whose lesions appear to lie in proteins; (iii) the demonstration of recognition sites in the bacterial cytoplasmic membrane for uncoupling molecules (Ordal, 1976; Brummett & Ordal, 1977; Nicholas & Ordal, 1978).

An alternative view of uncoupling that is consistent both with the observed effects of uncouplers generally (see above) and with the uncoupler and double-inhibitor titrations obtained in the present work is that the binding of uncouplers to proteinaceous components in energy-coupling membranes leads in some manner to a co-operative and uncoupling conformational transition in the energy-coupling membrane (Weinbach & Garbus, 1969)

that does not take place in the absence of uncoupler. Such a conclusion is also required to account for the mode of uncoupling by membrane-active bacteriocins (Kell *et al.*, 1981; Kell & Morris, 1981).

Such considerations lead to the view that the processes of energy coupling and their uncoupling are not related to each other in a simple fashion.

Venturoli & Melandri (1982) have recently extended their work on double-inhibitor titrations of chromatophores from *Rps. sphaeroides*, and conclude, in harmony with the present work, that the energetic coupling between the redox reactions and phosphorylation is localized in nature.

D. B. K. thanks Dr. S. J. Ferguson, Dr. H. V. Westerhoff and, in particular, Dr. J. B. Jackson for many lively and stimulating discussions on the content of this article. This work was supported by the Science and Engineering Research Council, U.K.

References

Armitage, J. P. & Evans, M. C. W. (1981) *FEBS Lett.* **126**, 98–102

Baccarini-Melandri, A., Casadio, R. & Melandri, B. A. (1977) *Eur. J. Biochem.* **78**, 389–402

Baccarini-Melandri, A., Casadio, R. & Melandri, B. A. (1981) *Curr. Top. Bioenerg.* **12**, 197–258

Baum, H., Hall, G. S., Nalder, J. & Beechey, R. B. (1971) in *Energy Transduction in Respiration and Photosynthesis* (Quagliariello, E., Papa, S. & Rossi, C. S., eds.), pp. 747–755, Adriatica Editrice, Bari

Brummett, T. B. & Ordal, G. W. (1977) *Arch. Biochem. Biophys.* **178**, 368–372

Casadio, R., Baccarini-Melandri, A. & Melandri, B. A. (1978) *FEBS Lett.* **87**, 323–328

Casadio, R., Venturoli, G. & Melandri, B. A. (1981) *Photobiochem. Photobiophys.* **2**, 245–253

Cirillo, V. P. & Gromet-Elhanan, Z. (1981) *Biochim. Biophys. Acta* **636**, 244–253

Clark, A. J. & Jackson, J. B. (1981) *Biochem. J.* **200**, 389–397

Clayton, R. K. (1963) *Biochim. Biophys. Acta* **75**, 312–313

Clayton, R. K. & Sistrom, W. R. (1978) (eds.) *The Photosynthetic Bacteria*, Plenum Press, New York

Conover, T. E. & Azzone, G. F. (1981) in *Mitochondria and Microsomes* (Lee, C. P., Schatz, G. & Dallner, G., eds.), pp. 481–518, Addison-Wesley, New York

Crofts, A. R. (1974) in *Perspectives in Membrane Biology* (Estrada-O, S. & Gitler, C., eds.), pp. 373–412, Academic Press, London

Crofts, A. R. & Jackson, J. B. (1970) in *Electron Transport and Energy Conservation* (Tager, J. M., Papa, S., Quagliariello, E. & Slater, E. C., eds.), pp. 383–408, Adriatica Editrice, Bari

Crofts, A. R. & Wood, P. M. (1978) *Curr. Top. Bioenerg.* **7**, 175–244

Decker, S. J. & Lang, D. R. (1977) *J. Biol. Chem.* **252**, 5936–5938

Decker, S. J. & Lang, D. R. (1978) *J. Biol. Chem.* **253**, 6738–6743

Del Valle-Tascon, S., van Grondelle, R. & Duysens, L. N. M. (1978) *Biochim. Biophys. Acta* **504**, 26–39

Elema, R. P., Michels, P. A. M. & Konings, W. N. (1978) *Eur. J. Biochem.* **92**, 381–387

Ferguson, S. J., Jones, O. T. G., Kell, D. B. & Sorgato, M. C. (1979) *Biochem. J.* **180**, 75–85

Gromet-Elhanan, Z. (1977) *Trends Biochem. Sci.* **2**, 274–277

Guffanti, A. A., Blumenfeld, H. & Krulwich, T. A. (1981) *J. Biol. Chem.* **256**, 8416–8421

Hanstein, W. G. (1976*a*) *Biochim. Biophys. Acta* **456**, 129–148

Hanstein, W. G. (1976*b*) *Trends Biochem. Sci.* **1**, 65–67

Hatefi, Y. (1975) *J. Supramol. Struct.* **3**, 201–213

Ito, M. & Ohnishi, Y. (1981) *FEBS Lett.* **136**, 225–230

Jackson, J. B. & Clark, A. J. (1981) in *Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria* (Palmieri, F., Quagliariello, E., Siliprandi, N. & Slater, E. C., eds.), pp. 371–379, Elsevier/North-Holland, Amsterdam

Jackson, J. B., Greenrod, J. A., Packham, N. K. & Petty, K. M. (1978) in *Frontiers of Biological Energetics* (Dutton, P. L., Leigh, J. S. & Scarpa, A., eds.), pp. 316–325, Academic Press, New York

Jackson, J. B., Venturoli, G., Baccarini-Melandri, A. & Melandri, B. A. (1981) *Biochim. Biophys. Acta* **636**, 1–8

Katre, N. V. & Wilson, D. F. (1978) *Arch. Biochem. Biophys.* **191**, 647–656

Kell, D. B. (1979) *Biochim. Biophys. Acta* **549**, 55–99

Kell, D. B. (1981) *Trends Biochem. Sci.* **7**, 1–2

Kell, D. B. & Morris, J. G. (1981) in *Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria* (Palmieri, F., Quagliariello, E., Siliprandi, N. & Slater, E. C., eds.), pp. 339–347, Elsevier/North-Holland, Amsterdam.

Kell, D. B., Ferguson, S. J. & John, P. (1978) *Biochim. Biophys. Acta* **502**, 111–126

Kell, D. B., Ferguson, S. J. & John, P. (1979) *Biochem. Soc. Trans.* **6**, 1292–1295

Kell, D. B., Clarke, D. J. & Morris, J. G. (1981) *FEMS Microbiol. Lett.* **11**, 1–11

Lienhard, G. E. & Secemski, I. I. (1973) *J. Biol. Chem.* **248**, 1121–1123

Margolis, S. A., Lenaz, G. & Baum, H. (1967) *Arch. Biochem. Biophys.* **118**, 224–230

McLaughlin, S. G. A. & Dilger, J. P. (1980) *Physiol. Rev.* **60**, 825–863

Melandri, B. A., Venturoli, G., de Santis, A. & Baccarini-Melandri, A. (1980) *Biochim. Biophys. Acta* **592**, 38–52

Melandri, B. A., Baccarini-Melandri, A. & Venturoli, G. (1981) in *Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria* (Palmieri, F., Quagliriello, E., Siliprandi, N. & Slater, E. C., eds.), pp. 381–388, Elsevier/North-Holland, Amsterdam

Michels, P. A. M. & Konings, W. N. (1978) *Eur. J. Biochem.* **85**, 147–155

Mitchell, P. (1966) *Biol. Rev.* **41**, 445–502

Mitchell, P. (1979*a*) *Eur. J. Biochem.* **95**, 1–20

Mitchell, P. (1979*b*) *Science* **206**, 1148–1159

Nicholas, R. A. & Ordal, G. W. (1978) *Biochem. J.* **176**, 639–647
Nishimura, M., Ito, T. & Chance, B. (1962) *Biochim. Biophys. Acta* **50**, 177–182
Ordal, G. W. (1976) *J. Bacteriol.* **126**, 72–79
Ostroumov, S. A., Jasaitis, A. A. & Samuilov, V. D. (1979) in *Biomembranes* (Manson, L. A., ed.), vol. 10, pp. 209–233, Plenum Press, New York
Packham, N. K., Berriman, J. A. & Jackson, J. B. (1978) *FEBS Lett.* **89**, 205–210
Petty, K. M. & Jackson, J. B. (1979*a*) *Biochim. Biophys. Acta* **547**, 463–473
Petty, K. M. & Jackson, J. B. (1979*b*) *Biochim. Biophys. Acta* **547**, 474–483
Reed, D. W. & Raveed, D. (1972) *Biochim. Biophys. Acta* **283**, 79–91
Saphon, S., Jackson, J. B., Lerbs, V. & Witt, H. T. (1975) *Biochim. Biophys. Acta* **408**, 58–66
Terada, H. & van Dam, K. (1975) *Biochim. Biophys. Acta* **387**, 507–518
Venturoli, G. & Melandri, B. A. (1982) *Biochim. Biophys. Acta* **680**, 8–16
Weinbach, E. C. & Garbus, J. (1969) *Nature (London)* **221**, 1016–1018
Wikström, M. & Krab, K. (1979) *Biochim. Biophys. Acta* **549**, 177–222
Williams, R. J. P. (1978) *FEBS Lett.* **85**, 9–19