

Charge screening by cations affects the conformation of the mitochondrial inner membrane

A study of exogenous NAD(P)H oxidation in plant mitochondria

Ian M. MØLLER and John M. PALMER

Department of Botany, Imperial College of Science and Technology, Prince Consort Road, London SW7 2BB, U.K.

(Received 4 November 1980/Accepted 20 February 1981)

Cations caused a decrease in the apparent K_m and an increase in the V_{max} for the oxidation of exogenous NADH by both Jerusalem-artichoke (*Helianthus tuberosus*) and *Arum maculatum* (cuckoo-pint) mitochondria prepared and suspended in a low-cation medium ($\approx 1 \text{ mM-K}^+$). In *Arum* mitochondria the addition of cations caused a much greater stimulation of the oxidation of NAD(P)H via the cytochrome oxidase pathway than via the alternative, antimycin-insensitive, pathway. This shows that cations affected a rate-limiting step in the electron-transport chain at or beyond ubiquinone, the branch-point of electron transport in plant mitochondria. The effects were only dependent on the valency of the cation (efficiency $\text{C}^{3+} > \text{C}^{2+} > \text{C}^+$) and not on its chemical nature, which is consistent with the theory of the diffuse layer. The results are interpreted to show that the screening of fixed negative membrane charges on lipids and protein complexes causes a conformational change in the mitochondrial inner membrane, leading to a change in a rate-limiting step of NAD(P)H oxidation. More specifically, it is proposed that screening removes electrostatic restrictions on lateral diffusion and thus accelerates diffusion-limited steps in electron transport.

Fixed charges on biological membranes have a major effect on the kinetic parameters of membrane-bound enzymes, since the concentration of a charged molecule in the diffuse layer associated with the membranes can be quite different from that in the bulk of the solution (Goldstein *et al.*, 1964; Wharton *et al.*, 1968; Hornby *et al.*, 1968; Engasser & Horvath, 1975; Maurel & Douzou, 1976; Douzou & Maurel, 1977; Wojtczak & Nalecz, 1979). Increasing ionic strength can affect the degree of screening and the local pH and thus alter the apparent pH optimum of membrane-bound enzymes (Goldstein *et al.*, 1964; Maurel & Douzou, 1976; Douzou & Maurel, 1977). The degree of charge screening will also alter the apparent K_m of membrane-bound enzymes for a charged substrate (Goldstein *et al.*, 1964; Wharton *et al.*, 1968). Wojtczak & Nalecz (1979) manipulated the surface charge

density of a series of biological membranes by adding positively or negatively charged surfactants and observed predictable effects on the apparent K_m of several enzymes bound to these membranes. They did not observe any influence of the altered surface charge on the apparent V_{max} of the enzymes measured. Johnston *et al.* (1979) reported that cations caused an unspecific stimulation of the oxidation of exogenous NADH by plant mitochondria. The dehydrogenase responsible for this oxidation is believed to be located on the outer surface of the inner membrane (Palmer & Passam, 1971). Since the effectiveness of cations to stimulate the oxidation of NADH was only dependent on the valency of the cation ($\text{C}^{3+} > \text{C}^{2+} > \text{C}^+$) and not on its identity [e.g. Ca^{2+} , Mg^{2+} and $(\text{DM})^{2+}$ had equal effect], it was suggested that the cations were effective by screening the fixed negative charges on the membrane by reducing the electrical potential, thus allowing the negatively charged NADH to reach more rapidly the active site of the membrane-bound dehydrogenase (Johnston *et al.*, 1979). The present data show the influence of the concentration of cations on the kinetic parameters of the

Abbreviations used: (DM)Br₂, decamethylenebis(trimethylammonium) bromide; (DM)²⁺, the cation formed by the dissociation of (DM)Br₂; Tes, 2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]ethanesulphonic acid.

NADH dehydrogenase in order to test the above hypothesis.

It was found that the addition of cations changes both the K_m and the V_{max} of NADH oxidation in two different types of plant mitochondria. Furthermore, in *Arum maculatum* (cuckoo-pint) mitochondria, which contain high activities of cyanide-resistant oxidase, the presence of cations alters the apparent capacity of the rival oxidases for available electrons. The results are interpreted to show that the degree of screening of fixed charges on membranes can affect not only the ability of substrates to reach membrane-bound enzymes, but also appear to affect the conformation of the membrane, resulting in an altered catalytic potential of the enzymes. A mechanism by which screening of charges might affect electron transport is proposed.

Materials and methods

Isolation of mitochondria

Mitochondria from Jerusalem artichoke (*Helianthus tuberosus*) tubers and *A. maculatum* spadices were isolated as described by Palmer & Kirk (1974) and Cammack & Palmer (1977), with the modification described by Møller *et al.* (1981a). In all cases the mitochondria were finally suspended in a low-salt medium ($\sim 1 \text{ mM-K}^+$) to maximize the effect of subsequent cation additions.

Oxidation of NAD(P)H

NAD(P)H oxidation was measured either as O_2 consumption in a Rank Brothers oxygen electrode (1 ml, 25°C) or as the disappearance of NAD(P)H measured at 340 nm in an Aminco DW 2 spectrophotometer (1 ml, room temperature). The medium consisted of 0.3 M-sucrose, 5 mM- K^+ /Tes and 0.2 μM -carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, pH 7.2, unless otherwise noted. Additions are specified in the legends to the Figures and Tables. The reaction was started by the addition of NAD(P)H. The actual concentration of NAD(P)H in the stock solution was determined with lactate dehydrogenase/pyruvate.

Determination of protein concentration

Protein was determined by the method of Lowry *et al.* (1951), after solubilization with 0.5% deoxycholate. Bovine serum albumin was used as the standard.

Chemicals

NAD(P)H was from Boehringer, antimycin A from Calbiochem, (DM)Br₂ from Sigma and tris(ethylenediamine)cobalt(III) chloride from Alfa Products, Danver, MA 01423, U.S.A. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and *m*-chlorobenzhydroxamic acid (synthesized as de-

scribed by Renfrow & Hauser, 1937) were generously given by Dr. P. G. Heytler (Du Pont Chemicals), and Dr. A. Bergman and Dr. I. Ericson (Department of Biochemistry, University of Umeå, Umeå, Sweden), respectively.

Results

Effect of cations on the apparent K_m and V_{max} of NADH oxidation

Fig. 1 shows that cations stimulate NADH oxidation by *Arum* mitochondria. (DM)²⁺ was used as a screening cation because it is not likely to be naturally occurring and because it binds or complexes very weakly. [The tetramethylammonium ion,

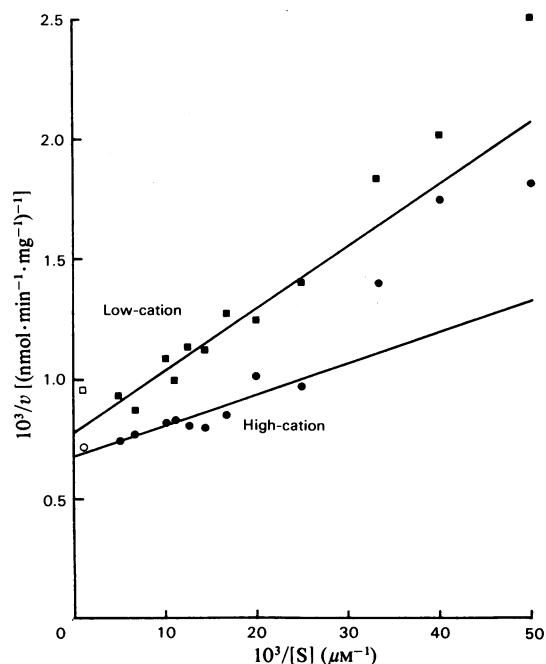


Fig. 1. Lineweaver-Burk plot of the effect of cations on NADH oxidation in *Arum* mitochondria

NADH oxidation was measured at 340 nm (86 μg of mitochondrial protein) in the low-salt medium as described in the Materials and methods section in the absence (■) or in the presence (●) of 2 mM-(DM)Br₂. The results from oxygen-electrode experiments on the same preparation of mitochondria (1 mM-NADH) are also indicated: low-salt conditions (□) and high-salt conditions (○). Rates are given as nmol of NADH $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ or nmol of $\text{O} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. The lines are fitted by the least-squares method, the points at the three lowest NADH concentrations being left out. V_{max} and K_m were $1.29 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ and $33 \mu\text{M}$ for low-cation conditions and $1.48 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ and $19 \mu\text{M}$ for high-cation conditions.

which is similar in structure to the charged groups of $(DM)^{2+}$, is assumed to have a much smaller binding constant than Na^+ (Nir & Bentz, 1978.) From previous work we know that $2mM-(DM)^{2+}$ gives maximal screening (Johnston *et al.*, 1979; Møller *et al.*, 1981*a,b*). The oxidation always showed a 'roll-on', that is, a maximum linear rate was only observed after a lag, and this resulted in an underestimate of the rates at very low concentrations of NADH, since the NADH was exhausted before the maximal rate was reached. The problem of 'roll-on' has been discussed by Møller *et al.* (1981*b*). However, higher concentrations of NADH gave reliable data. The V_{max} estimated from the regression lines corresponds well to the maximal rate of oxygen consumption measured in the oxygen electrode at $1mM-NADH$ on the same preparation of mitochondria (Fig. 1).

The data for the apparent K_m and V_{max} from Fig. 1 are shown in Table 1, together with results from

similar experiments on Jerusalem-artichoke mitochondria. In both cases the addition of cations caused a significant decrease in the apparent K_m for NADH and an increase in the V_{max} . (Table 1). However, the apparent K_m values are so low that $1mM-NADH$ is saturating even under low-salt conditions. The stimulation by cations observed by Johnston *et al.* (1979) could, therefore, not be due to the change in apparent K_m , since all measurements in that study were performed with $1mM-NADH$.

Effect of cations on the electron-transport pathways in *Arum* mitochondria

In the course of the NADH-oxidation experiments on *Arum* mitochondria, it was noticed that cations affected the rate of NADH oxidation to a greater extent in the presence of *m*-chlorobenzhydroxamic acid than in the presence of antimycin A. This is shown in Fig. 2, where $2mM-(DM)Br_2$ was used (compare traces D and E in particular);

Table 1. Effect of cations on the kinetic parameters of NADH oxidation

The oxidation of NADH was measured as $340nm$ in a DW 2 spectrophotometer. For assay conditions, see the Materials and methods section. To obtain high-cation conditions, $2mM-(DM)Br_2$ was added. An example of the determination of K_m and V_{max} is shown in Fig. 1. Data are given as $\bar{x} \pm s.d.$ (number of preparations).

Mitochondria from	Parameter	Low-cation conditions	High-cation conditions	Effect of cations
Jerusalem artichoke	K_m (μM)	91 (1)	54 (1)	-41%
	V_{max} ($nmol \cdot min^{-1} \cdot mg^{-1}$)	395 (1)	555 (1)	+41%
<i>Arum maculatum</i>	K_m (μM)	31 ± 4 (2)	19 ± 1 (2)	$-36 \pm 9\%$ (2)
	V_{max} ($nmol \cdot min^{-1} \cdot mg^{-1}$)	1260 ± 50 (2)	1590 ± 160 (2)	$+29 \pm 19\%$ (2)

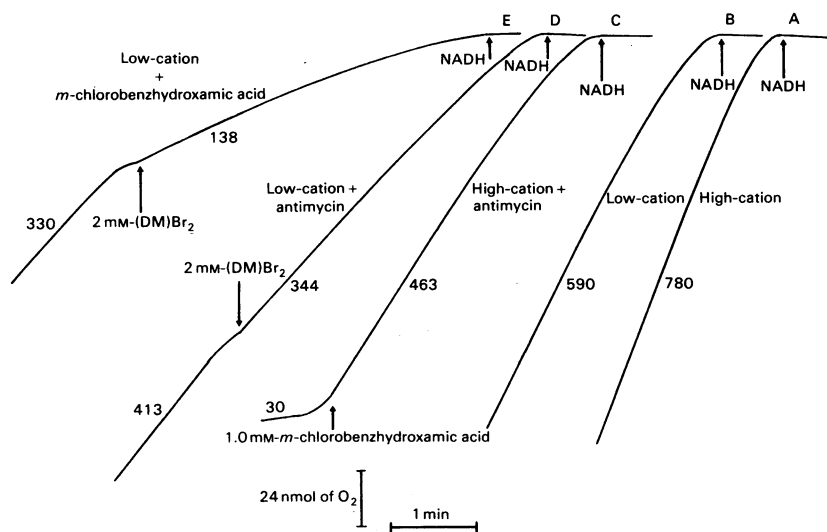


Fig. 2. Effect of cations on the two electron-transport pathways in *Arum* mitochondria

The oxidation of NADH was measured in an oxygen electrode as described in the Materials and methods section, with $0.13mg$ of mitochondrial protein $\cdot ml^{-1}$. The following additions were made to the basal low-cation medium before NADH ($1mM$): A, $2mM-(DM)Br_2$; B, none; C, $2mM-(DM)Br_2 + 0.36\mu M$ -antimycin A; D, $0.36\mu M$ -antimycin A; E, $1.0mM$ -*m*-chlorobenzhydroxamic acid. Numbers on traces indicate rates in $nmol$ of $O_2 \cdot min^{-1} \cdot mg^{-1}$.

however, NaCl (100 mM), MgCl₂ and CaCl₂ (2 mM) and tris(ethylenediamine)cobalt(III) chloride (100 μM) all had comparable effects. At these concentrations the cations have been shown to give maximal screening of fixed negative membrane charges at all concentrations of *Arum* mitochondria up to 0.5 mg · ml⁻¹ (Fig. 3 in Møller *et al.*, 1981a).

A detailed analysis of the effect of cations on the flux of electrons through the two oxidase systems is presented in Table 2. The maximal rate of NADH oxidation is clearly stimulated by cations, just as was the case in Jerusalem-artichoke mitochondria (Johnston *et al.*, 1979). The addition of either *m*-chlorobenzhydroxamic acid, inhibitor of the alternative oxidase (Schonbaum *et al.*, 1971), or antimycin A, inhibitor of the cytochrome oxidase pathway, caused an inhibition under both low- and high-cation conditions. Thus, in the presence of an inhibitor, neither pathway could accommodate the full, uninhibited, electron flow. This indicates that a rate-limiting step exists on either pathway somewhere between ubiquinone, the point at which the two electron-transport pathways separate (Henry &

Nyns, 1975; Storey, 1976), and oxygen. It also means that (B) and (C) in Table 2 represent estimates of the maximum capacities of the cytochrome oxidase and the alternative oxidase pathways respectively. Adding these (B + C; Table 2), one gets within 10% of the maximal uninhibited rate (A; Table 2) under both low- and high-cation conditions. Thus both pathways were also used at their maximal capacity in the absence of inhibitor, and no division of electrons occurred on addition of an inhibitor. However, the addition of cations caused a stimulation of NADH oxidation, and the greater proportion of this increase in electron flow beyond ubiquinone was carried by the cytochrome oxidase pathway (159 as against 70 nmol · min⁻¹ · mg⁻¹ for the alternative oxidase pathway). This stimulation changes the ratio between the maximal capacities of the two pathways quite significantly (Table 2).

Effect of cations on NADPH oxidation by Arum mitochondria

Arum mitochondria also oxidize exogenous NADPH, as has been shown for mitochondria from

Table 2. *Effect of cations on the two electron-transport pathways in Arum mitochondria*

The rate of NADH oxidation is given as nmol of O₂ · min⁻¹ · mg⁻¹ and was measured in 0.3 M-sucrose, 5 mM-Tes, pH 7.2, 0.2 μM-carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone and 1 mM-NADH (low-cation conditions). High-cation conditions were obtained by adding 2 mM-(DM)Br₂. Other additions were 1 mM-*m*-chlorobenzhydroxamic acid and 0.36 μM-antimycin A. Data are expressed as $\bar{x} \pm$ s.d. (number of preparations).

Inhibitors added	Low-cation conditions		High-cation conditions		Stimulation of rate by cations (nmol · min ⁻¹ · mg ⁻¹)
	Rate of NADH oxidation (nmol · min ⁻¹ · mg ⁻¹)	Percentage of 'uninhibited' rate	Rate of NADH oxidation (nmol · min ⁻¹ · mg ⁻¹)	Percentage of 'uninhibited' rate	
(A) None (control)	604 ± 82 (6)	100	785 ± 126 (6)	100	180 ± 50 (6)
(B) <i>m</i> -chlorobenzhydroxamic acid	140 ± 16 (5)	24 ± 2 (5)	297 ± 49 (5)	40 ± 5 (5)	159 ± 35 (5)
(C) Antimycin	410 ± 62 (5)	70 ± 4 (5)	480 ± 78 (5)	64 ± 4 (5)	70 ± 24 (5)
(D) <i>m</i> -chlorobenzhydroxamic acid + antimycin	37 ± 20 (3)	6 ± 2 (3)	38 ± 13 (5)	5 ± 2 (5)	4 ± 7 (3)
Ratio between two pathways (C/B)	2.9 ± 0.4 (5)		1.6 ± 0.3 (5)		

Table 3. *Effect of cations on NADH and NADPH oxidation in Arum mitochondria*

The oxidation of NAD(P)H was measured in medium containing 0.3 M-sucrose, 10 mM-Mops (4-morpholinepropane-sulphonic acid), 0.2 μM-carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, pH 6.7, with 0.12 mg of mitochondrial protein · ml⁻¹. The reaction was started with 1 mM-NAD(P)H. Other additions were 1 mM-*m*-chlorobenzhydroxamic acid and 0.36 μM-antimycin A. Rates are significantly higher than in Table 2, since the spadices were picked later in the season, closer to the climacteric (Meeuse, 1975).

Inhibitors	Rate (nmol of O ₂ · min ⁻¹ · mg ⁻¹) or percentage stimulation					
	NADH oxidation			NADPH oxidation		
	Low-cation conditions	High-cation conditions*	Stimulation by cations (%)	Low-cation conditions	High-cation conditions*	Stimulation by cations (%)
None (control)	902	1250	39	527	740	40
<i>m</i> -Chlorobenzhydroxamic acid	142	288	103	125	267	113
Antimycin	698	812	16	438	583	33
<i>m</i> -Chlorobenzhydroxamic acid + antimycin	31	38	—	31	42	—

* + 2 mM-(DM)Br₂.

a variety of other plant tissues (Arron & Edwards, 1979). In Table 3 the effects of cations on NADH and NADPH oxidation and the balance of the two pathways are compared at pH 6.7, the pH optimum for NADPH oxidation in *Arum* mitochondria (I. M. Møller, unpublished work). The results at pH 6.7 for both NADH and NADPH are similar to those for NADH at pH 7.2 shown in Table 2. It is worth noticing, however, that the two substrates give identical rates in the presence of *m*-chlorobenzhydroxamic acid, whereas NADH gives significantly higher rates than NADPH in the presence of antimycin. This observation suggests that NADH and NADPH are not oxidized via the same pathway. A similar conclusion has also been reached by Arron & Edwards (1979) and Koeppel & Miller (1972).

Discussion

In a previous paper (Johnston *et al.*, 1979) we reported that the ability of cations to stimulate NADH oxidation by plant mitochondria was only dependent on the charge of the cation and independent of its chemical nature. It was suggested that cations screened the surface charges and facilitated the approach of the negatively charged substrate to the membrane-bound enzyme. This hypothesis has been shown to be partly correct, since the present data show that the presence of cations does decrease the apparent K_m of the mitochondria for the NADH (Fig. 1, Table 1). However, since the apparent K_m is so low, it seems that the 1 mM-NADH normally used saturates the enzyme. Consequently, the change in K_m cannot explain the observed stimulation of NADH oxidation. The data presented in Table 1 show that there is also a change in the apparent V_{max} , which could be more significant than the change in K_m . A change in V_{max} for a chain of reactions like that of $\text{NADH} \rightarrow \text{O}_2$ only indicates that the rate-limiting step is affected, but provides no evidence for where that rate-limiting step may be located.

The branch-point of the two respiratory pathways present in *Arum* mitochondria is located at ubiquinone (Henry & Nyns, 1975; Storey, 1976). Any factor that affects the maximal rate of electron flow along a pathway must be attributed to a change in the rate-limiting step exclusively linked to that pathway and therefore located between ubiquinone and the terminal oxidase of that pathway. The data in Tables 2 and 3 clearly show that the maximal rates along both pathways were affected by cations. Furthermore, the data demonstrate that cations differentially affect the rate-limiting steps of the two electron-transport pathways by favouring enhanced flux through the cytochrome oxidase branch (Fig. 2; Tables 2 and 3).

The mechanism by which this cation stimulation

takes place is obviously of some interest. It is not dependent on the chemical identity of the cation, only on its valency, and the order of efficiency is $\text{C}^{3+} > \text{C}^{2+} > \text{C}^+$ (Johnston *et al.*, 1979; the present paper). These properties are consistent with the Gouy-Chapman theory of the diffuse layer (see, e.g., Searle *et al.*, 1977), where cations are attracted to the negatively charged membranes, are concentrated in the diffuse layer adjacent to the membranes and cause a screening of the membrane charges. In chloroplasts, screening phenomena have been frequently observed (see Barber, 1980, for a review), but although thylakoid membranes contain 20–30% of charged lipids, sulpholipids, phosphatidylglycerol and phosphatidic acid (Nishihara *et al.*, 1980), the hypothesis proposed by Barber (1980) to explain these phenomena attributes the charge screening effects to charges on proteins only (Nakatani *et al.*, 1978). The inner membrane of plant (and mammalian) mitochondria also contains a relatively high proportion of charged lipids, here mainly in the form of diphosphatidylglycerol (McCarty *et al.*, 1973). No information is available on the contribution of proteins to the net negative charge (Møller *et al.*, 1981a) of plant mitochondrial membranes. It seems reasonable, however, to assume that both lipids and proteins contribute to the negative charge on the surface of the membrane.

Under low-cation conditions where the degree of screening is low, negatively charged molecules on the membrane will repel each other. This will have the effect of decreasing lateral diffusion, increasing the effective path length between collisions of enzyme complexes and giving a greater rigidity to the lipid bilayer, since the lipid head-groups will be kept as far separated as possible. The addition of cations will now remove these restrictions on mobility, e.g. cause a 'melting'-like change in the fluidity of the lipid phase, and an increase in turnover of the respiratory chain will follow, since this has been shown to be diffusion-dependent (Schneider *et al.*, 1980). Such a model could explain the results obtained in the present study.

Finally, it is worth comparing the kinetic properties of NADH oxidation in Jerusalem artichoke and *Arum* mitochondria, since these two types of mitochondria have been shown to have very different electrostatic properties (Møller *et al.*, 1981a). The results are summarized in Table 4. The qualitative effect of cations is the same in the two kinds of mitochondria: K_m is decreased and V_{max} is increased. Quantitatively, there are very significant differences: Jerusalem-artichoke mitochondria oxidize NADH five times slower than *Arum* mitochondria, have a K_m that is three times as high and are stimulated twice as much (in percentage terms) as *Arum* mitochondria by cations. All of these observations are consistent with the findings that

Table 4. *Properties of NADH oxidation correlated with the number of membrane charges*
All results are $\bar{x} \pm$ s.d. (number of preparations).

	Mitochondria from ... <i>Arum maculatum</i>	Jerusalem artichoke
(a) NADH oxidation in low-cation medium (nmol of O ₂ · min ⁻¹ · mg ⁻¹)	604 ± 82 (6)*	120 ± 12 (10)
(b) Stimulation by cations (%)	30 ± 6 (6)*	62 ± 25 (9)
(c) Apparent K _m (low-cation medium)	31 ± 4 μM (2)†	91 μM (1)†
No. of charges/mg of protein	Low‡	High‡

* From Table 2.

† From Table 1.

‡ From Møller *et al.* (1981a).

Jerusalem-artichoke mitochondria contain more negative charges per mg of protein than do *Arum* mitochondria (Table 4; Møller *et al.*, 1981a). This indicates that charges on membranes may have an important control function in NADH oxidation.

We thank Mrs. Jill Farmer for expert technical assistance. I. M. M. is the recipient of a NATO Science Fellowship and grants nos. 511-15019 and 511-20033 from the Danish Natural Science Research Council. This study was supported by grants from the Science Research Council, The Royal Society, and the Central Research Fund, University of London.

Note added in proof

In support of the proposal above that screening accelerates diffusion-limited steps in electron transport, Wojtczak & Nalecz (1979), who worked on the activity of individual enzymes, found no change in V_{\max} with changes in surface charge density.

References

- Arron, G. P. & Edwards, G. E. (1979) *Can. J. Biochem.* **57**, 1392–1399
- Barber, J. (1980) *FEBS Lett.* **118**, 1–10
- Cammack, R. & Palmer, J. M. (1977) *Biochem. J.* **166**, 347–355
- Douzou, P. & Maurel, P. (1977) *Trends Biochem. Sci.* **2**, 14–17
- Engasser, J.-M. & Horvath, C. (1975) *Biochem. J.* **145**, 431–435
- Goldstein, L., Levin, Y. & Katchalski, E. (1964) *Biochemistry* **3**, 1913–1919
- Henry, M.-F. & Nyns, E.-J. (1975) *Sub-Cell. Biochem.* **4**, 1–65
- Hornby, W. E., Lilly, M. D. & Crook, E. M. (1968) *Biochem. J.* **107**, 669–674
- Johnston, S. P., Møller, I. M. & Palmer, J. M. (1979) *FEBS Lett.* **108**, 28–32
- Koeppel, D. E. & Miller, R. J. (1972) *Plant Physiol.* **49**, 353–357
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maurel, P. & Douzou, P. (1976) *J. Mol. Biol.* **102**, 253–264
- McCarty, R. E., Douce, R. & Benson, A. A. (1973) *Biochim. Biophys. Acta* **316**, 266–270
- Meeuse, B. J. D. (1975) *Annu. Rev. Plant Physiol.* **26**, 117–126
- Møller, I. M., Chow, W. S., Palmer, J. M. & Barber, J. (1981a) *Biochem. J.* **193**, 37–46
- Møller, I. M., Johnston, S. P. & Palmer, J. M. (1981b) *Biochem. J.* **194**, 487–495
- Nakatani, H. Y., Barber, J. & Forrester, J. A. (1978) *Biochim. Biophys. Acta* **504**, 215–225
- Nir, S. & Bentz, J. (1978) *J. Colloid Interface Sci.* **65**, 399–414
- Nishihara, M., Yokota, K. & Kito, M. (1980) *Biochim. Biophys. Acta* **617**, 12–19
- Palmer, J. M. & Kirk, B. I. (1974) *Biochem. J.* **140**, 79–86
- Palmer, J. M. & Passam, H. C. (1971) *Biochem. J.* **122**, 16P–17P
- Renfrow, W. B., Jr. & Hauser, C. R. (1937) *J. Am. Chem. Soc.* **59**, 2308–2314
- Schneider, H., Lemasters, J. J., Höchli, M. & Hackenbrock, C. R. (1980) *J. Biol. Chem.* **255**, 3748–3756
- Schonbaum, G. R., Bonner, W. D., Storey, B. T. & Bahr, J. T. (1971) *Plant Physiol.* **47**, 124–128
- Searle, G. F. W., Barber, J. & Mills, J. D. (1977) *Biochim. Biophys. Acta* **461**, 413–425
- Storey, B. T. (1976) *Plant Physiol.* **58**, 521–525
- Wharton, C. W., Crook, E. M. & Brocklehurst, K. (1968) *Eur. J. Biochem.* **6**, 572–578
- Wojtczak, L. & Nalecz, M. J. (1979) *Eur. J. Biochem.* **94**, 99–107