Electrostatic screening stimulates rate-limiting steps in mitochondrial electron transport

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The effect of electrostatic screening of fixed negative charges on uncoupled mitochondrial electron transport was investigated with substrates with different charge and different sites of donation of electrons to the electron-transport chain of Jerusalem-artichoke (*Helianthus tuberosus* L.) mitochondria. Duroquinol (neutral substrate) was oxidized with a pH optimum of 7.6–7.8. The addition of cations caused a doubling of $V_{\max}$ (order of efficiency $C^{3+} > C^{2+} > C^{+}$) through electrostatic screening, whereas the K_m was unaffected. Screening stimulated (by 150%) the $V_{\max}$ for the oxidation of reduced cytochrome *c* (positive substrate; to O_2), but in this case the K_m doubled. The $V_{\max}$ of the oxidation of exogenous NADH (negative substrate) was also stimulated by screening when the acceptor was O_2 , but unaffected when duroquinone was the acceptor. In both cases, the K_m for NADH was considerably decreased. The effect of screening on the K_m for the different substrates can be explained by the changes in the effective concentration of substrate near the active site due to the lowering in the size of the surface potential. The effect of screening on the $V_{\max}$ of the different partial processes indicates that increasing the salt concentration of the medium enhances the maximal activity of cytochrome *c* oxidase. However, the results also point at the existence of other rate-limiting steps, which are affected by screening and may involve ubiquinone, in electron transport in plant mitochondria.

Schneider *et al.* (1980, 1982) have pin-pointed two rate-limiting steps in the electron-transport system of mammalian mitochondria. By fusing mitoplasts with liposomes they obtained several populations of mitoplasts that differed in their concentration of respiratory complexes. The partial reactions of electron transport from NADH to O_2 most affected by this dilution of complexes were those involving ubiquinone and cytochrome *c*. These reactions were therefore proposed to be diffusion rate-limiting in electron transport (Schneider *et al.*, 1980, 1982).

Abbreviations used: (DM)Br₂, *NNNN'*N'-hexamethyldecane-1,10-diamine bromide; duroquinol, tetramethyl-*p*-benzoquinol; duroquinone, tetramethyl-*p*-benzoquinone; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Mops, 4-morpholinepropane-sulphonic acid.

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In plant mitochondria the problem of rate-limiting steps has been approached from an entirely different angle. Added NADH is oxidized by these mitochondria and by mitochondria from fungi and other micro-organisms by a dehydrogenase located on the external surface of the inner membrane (see Palmer & Møller, 1982, for a brief review). A strong stimulation of this oxidation (to O_2) is observed as the cation content of the medium is increased. The stimulation is due to electrostatic screening of fixed negative charges on the mitochondrial membrane (Hackett, 1961; Johnston *et al.*, 1979; Møller & Palmer, 1981a; Møller *et al.*, 1982). We originally thought that the decreased negative surface potential (Møller *et al.*, 1981a) facilitated the approach of the negatively charged NADH to the membrane-bound enzyme and thus decreased the apparent K_m for NADH (Johnston *et al.*, 1979). Effects of changes in surface potentials on the K_m of membrane-bound enzymes have been reported repeatedly (see, e.g.,

Wojtczak & Nalecz, 1979; Nalecz *et al.*, 1980; Wojtczak *et al.*, 1982). However, increased screening affected not only the K_m of the reaction NADH-O_2 in plant mitochondria but also the $V_{\max}$. The rate of oxidation along the cytochrome *c* oxidase pathway was stimulated in *Arum* mitochondria, whereas the alternative oxidase pathway was unaffected (Møller & Palmer, 1981a). Since the two pathways are assumed to diverge at ubiquinone, a step between ubiquinone and O_2 seemed to be involved in changing the $V_{\max}$. (Henry & Nyns, 1975; Storey, 1976).

In the present work we have attempted to locate the partial reaction in mitochondrial electron transport affected by electrostatic screening. The $V_{\max}$ of the oxidation of NADH (negative substrate), duroquinol (neutral substrate) and cytochrome *c* (positive substrate) are all strongly increased by screening when the receptor is O_2 . The $V_{\max}$ of the reaction NADH- duroquinone is unchanged by the salt concentration of the medium. It is concluded that cytochrome *c* oxidase is stimulated by electrostatic screening, but that other rate-limiting steps are involved when longer segments of the chain are used.

Materials and methods

Isolation of mitochondria

Mitochondria were prepared from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers in a low-salt medium containing 0.3M-sucrose as described by Møller *et al.* (1981a). When these mitochondria were suspended in a medium containing only 0.02M-sucrose to rupture the outer membrane (Douce *et al.*, 1973), the rate of cytochrome *c* oxidation was 15-fold higher than in 0.3M-sucrose, indicating 93% integrity of the outer membrane.

Duroquinol oxidation

The oxidation of duroquinol (0.1–2.0mM as indicated) was measured in a Rank Brothers oxygen electrode at 25°C in a total volume of 1.0ml. The medium is described in the legends to Figures and Tables.

Cytochrome *c* oxidation

The oxidation of reduced cytochrome *c* (3–75 μM , prepared as described by Møller & Palmer, 1982) was measured in the presence of only 0.02M-sucrose (to rupture the outer membrane, see above) at 550–540nm in an Aminco DW2 dual-wavelength spectrophotometer at room temperature in a total volume of 1.0ml. The total composition of the medium is described in the legends to the Figures.

NADH oxidation

The rate of NADH oxidation by duroquinone was monitored spectrophotometrically at 340nm

at room temperature in a total volume of 1.0ml. The concentration of NADH was varied and the reaction was started by the addition of 0.3mM-duroquinone (final concentration). The total composition of the medium is described in the legend to Fig. 5.

Protein determination

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard, after solubilizing the mitochondria in 0.5% (w/v) sodium deoxycholate.

Chemicals

Duroquinol from K & K, ICN Pharmaceuticals, Plainview, NY, U.S.A., was dissolved in dimethyl sulphoxide just before use (except for Fig. 1; see the main text). The concentration of the stock solution (about 100mM) was determined by increasing the pH by the addition of 0.1M-KOH (final concentration), which causes the duroquinol to auto-oxidize. An absorption coefficient of $12.5\text{mM}^{-1}\cdot\text{cm}^{-1}$ for $A_{265(\text{red.})} - A_{265(\text{ox.})}$ was used (Lawford & Garland, 1972).

Cytochrome *c* was from Boehringer; EGTA, (DM) Br_2 , mersalyl, rotenone and chlortetracycline were from Sigma (London) Chemical Co.; tris(ethylenediamine)Co(III) chloride was from Alfa Products, Danvers, MA, U.S.A. (U.K. distributors: Lancaster Synthesis, Lancaster, U.K.); antimycin A was from Calbiochem, Lucerne, Switzerland; FCCP was a gift from Dr. P. G. Heytler, Du Pont Chemicals, and *m*-chlorobenzhydroxamic acid a gift from Dr. A. Bergman and Dr. I. Ericson, Department of Biochemistry, University of Umeå, Sweden.

The rest of the chemicals were from BDH Chemicals, Poole, Dorset, U.K., Hopkin and Williams, Chadwell Heath, Essex, U.K., or Sigma Chemical Co. and were of the highest grade available.

Results and discussion

The partial reactions investigated were (1) NADH-O_2 (Johnston *et al.*, 1979; Møller & Palmer, 1981a), (2) duroquinol-O_2 , (3) $\text{duroquinol-oxidized cytochrome } c$, (4) $\text{reduced cytochrome } c\text{-O}_2$ and (5) NADH- duroquinone (numbers refer to schematic drawing in Scheme 1 and the results are presented in this order).

Duroquinol oxidation

Before the use of duroquinol oxidation (reaction 2, Scheme 1) in kinetic measurements, the pH optimum was determined. After reaching an optimum at pH 7.8 the rate of duroquinol oxidation decreased at pH 8.0, only to increase dramatically

Scheme 1. Schematic representation of the different partial reactions investigated in the electron-transport chain of Jerusalem-artichoke mitochondria

We do not know the exact sites of interaction of reduced and oxidized duroquinone (DHQ). For convenience, we let both of them interact with the ubiquinone pool (UQ). Other abbreviation: Cyt., cytochrome.

Fig. 1. pH-dependence of duroquinol oxidation by Jerusalem-artichoke mitochondria

The oxidation was measured by suspending mitochondria at 0.30 mg of protein/ml in medium containing 0.3 M-sucrose, 0.1 M-Mops buffer, 2 mM-(DM)Br₂ and 0.2 μ M-FCCP with (●) or without (□) 1.9 μ M-antimycin A at the pH indicated and measuring the rate of O₂ consumption upon addition of 2 mM-duroquinol (final concentration). The antimycin A-sensitive part of the oxidation is also shown (■). A 1–2 h-old duroquinol solution was used at pH 6.0–7.8 and a fresh one at pH 7.8–8.4 (see the text).

at pH 8.2 and 8.4 (Fig. 1). In the presence of antimycin A to inhibit mitochondrial electron transport, duroquinol oxidation increased exponentially with increasing pH. The antimycin A-insensitive oxidation of duroquinol was also observed in the absence of mitochondria (results not shown), and was thus due to chemical reduction of O₂ by duroquinol at alkaline pH. The pH optimum of the antimycin A-sensitive oxidation of duroquinol was at pH 7.6–7.8 (Fig. 1). At pH 7.2 that rate was 85% of the maximum, and, since this pH has been used

in our previous investigations (Johnston *et al.*, 1979; Møller & Palmer, 1981a) and the interference from chemical oxidation of duroquinol was minimal, pH 7.2 was used in subsequent experiments. Fig. 1 also illustrates the importance of using freshly prepared solutions of duroquinol, as a 1–2 h-old solution gave much higher antimycin A-insensitive oxidation than did a freshly prepared one.

Duroquinol oxidation was insensitive to EGTA, EDTA, chlortetracycline + CaCl₂ and mersalyl, all of which inhibit NADH oxidation strongly (Table 1; Coleman & Palmer, 1971; Cowley & Palmer, 1978; Møller & Palmer, 1981b; Møller *et al.*, 1981b; Palmer & Møller, 1982; Møller *et al.*, 1983). Antimycin A inhibited the oxidation of both substrates completely, whereas rotenone had only a marginal effect (Table 1). The results show that duroquinol donated electrons either to the quinone pool or directly to the cytochrome *b-c*₁ complex but before the antimycin A block. The partial inhibition of duroquinol oxidation by *m*-chlorobenzhydroxamic acid is consistent with the suggestion by Rich *et al.* (1978) that substituted benzhydroxamic acids can act as competitive inhibitors in enzyme reactions involving quinones.

The addition of cations to mitochondria suspended in a low-salt medium strongly stimulated duroquinol oxidation (Fig. 2). The concentration at which uni-, bi- and ter-valent cations were efficient (10–100 μ M, 0.1–5 mM and 10–100 mM respectively) is the same as that observed with NADH oxidation (Johnston *et al.*, 1979; Møller *et al.*, 1981a, 1982) and is indicative of electrostatic screening. Cations stimulated the oxidation by 100–200% (Fig. 2), and this was the result of an increased *V*_{max} (Fig. 3). The *K*_m was 0.53 and 0.45 mM in the presence and in the absence of 100 mM-KCl respectively (Fig. 3).

An attempt to measure mitochondrial electron transport from duroquinol to oxidized cytochrome *c* (reaction 3, Scheme 1) at pH 7.2 was unsuccessful.

Table 1. *Effect of inhibitors on NADH and duroquinol oxidation by Jerusalem-artichoke mitochondria*

The reaction medium contained 0.3M-sucrose, 0.1M-Mops/KOH buffer, pH 7.2, 2mM-(DM)Br₂, 0.2 μ M-FCCP and 0.3–0.5mg of mitochondrial protein/ml plus inhibitors as indicated. The reaction was started by the addition of 1mM-NADH or 1mM-duroquinol (final concentration). No correction was made for antimycin A-insensitive duroquinol oxidation. Control rates were 172 and 237nmol of O₂/min per mg for NADH oxidation and 188, 208 and 382nmol of O₂/min per mg for duroquinol oxidation. Values are means $\pm$ s.d. (numbers of independent preparations in parentheses).

Inhibitor	Inhibition (%)	
	Of NADH oxidation	Of duroquinol oxidation
EGTA (1mM)	88 (1)	4 $\pm$ 4 (3)
EDTA (1mM)	91 (1)	8 $\pm$ 3 (3)
Chlortetracycline (50 μ M)	9 (1)	12 $\pm$ 8 (2)
Chlortetracycline (50 μ M) + CaCl ₂ (2mM)	82 (1)	15 $\pm$ 8 (3)
Mersalyl (20 μ M)	80 $\pm$ 6 (2)	26 $\pm$ 4 (2)
Rotenone (20 μ M)	17 $\pm$ 6 (2)	15 $\pm$ 6 (2)
<i>m</i> -Chlorobenzhydroxamic acid (1mM)	5 $\pm$ 4 (2)	31 $\pm$ 8 (2)
Antimycin A (0.36 μ M)	> 97 (1)	> 95 (2)

Fig. 2. *Stimulation of duroquinol oxidation by Jerusalem-artichoke mitochondria through addition of salts*

The reaction medium contained 0.3M-sucrose, 0.1M-Mops/KOH buffer, pH 7.2, 2mM-(DM)Br₂, 0.2 μ M-FCCP and of mitochondrial protein/ml, 1.0mM-duroquinol and salts as indicated. Abbreviation: (TEC)Cl₃, tris(ethylenediamine)Co(III) chloride. The control rate in the absence of added salts was 134nmol/min per mg.

A very rapid antimycin A-insensitive cytochrome *c* reduction was observed. This is consistent with the observation by Rich & Bendall (1980) that duroquinol will reduce cytochrome *c* non-enzymically at neutral pH.

Oxidation of reduced cytochrome c

The oxidation of reduced cytochrome *c* (reaction 4, Scheme 1) was also stimulated by cations through an increased $V_{\max}$, but, unlike the results with duroquinol, the K_m for cytochrome *c* was increased by screening (Fig. 4). The increase in $V_{\max}$ with increased salt concentration is in contrast with results obtained with cytochrome *c* oxidase from mammalian tissues, where either no effect (Smith *et al.*, 1981) or a decrease (Douzou &

Maurel, 1976) has been reported at neutral pH. The reason for this difference between plant and mammalian cytochrome *c* oxidases is not known, as we have very little information about the enzyme from plant sources (Bomhoff & Spencer, 1977).

Oxidation of NADH

When O₂ is the ultimate electron acceptor (reaction 1, Scheme 1), the $V_{\max}$ of NADH oxidation is strongly stimulated by screening, whereas the K_m is decreased (Johnston *et al.*, 1979; Møller & Palmer, 1981a). When the acceptor is duroquinone, the K_m for NADH is also decreased by screening. However, in this case the $V_{\max}$ is practically unaffected (Fig. 5).

Fig. 3. Eadie-Hofstee plot of the kinetics of duroquinol oxidation by Jerusalem-artichoke mitochondria at low and high concentrations of cations

The medium contained 0.3M-sucrose, 5mM-Mops buffer, pH 7.2, 0.2 μ M-FCCP and 0.28 mg of mitochondrial protein/ml with (●) or without (○) 100mM-KCl. The reaction was started by the addition of 0.25–2.0mM-duroquinol (final concentration). The lines are fitted by linear-regression analysis. $V_{\max}$ is given in nmol of O_2 /min per mg.

Fig. 4. Eadie-Hofstee plot of the kinetics of cytochrome *c* oxidation by Jerusalem-artichoke mitochondria at low and high concentrations of cations

The medium contained 0.02M-sucrose (see the Materials and methods section), 5mM-Mops buffer, pH 7.2, and 0.2 μ M-FCCP with (●; 9.2 μ g of protein/ml) or without (○; 18 μ g of protein/ml) 100mM-KCl. The reaction was started by the addition of 1.5–75 μ M-reduced cytochrome *c* (final concentration) and the initial rate was determined. $V_{\max}$ is given in nmol of cytochrome *c*/min per mg.

Effect of screening on K_m of membrane-bound enzymes

Cations decrease the size of the negative surface potential through screening of the negative surface charges on biological membranes (Johnston *et al.*, 1979; Barber, 1980, 1982; Chow & Barber, 1980; Møller & Palmer, 1981a). The concentration of charged substrates near the membrane surface changes with changes in the surface potential, and the effect on the kinetic parameters of membrane-bound enzymes can be predicted (Douzou & Maurel, 1976; Maurel & Douzou, 1976; Wojtczak & Nalecz, 1979; Nalecz *et al.*, 1980). For enzymes with a positively charged substrate one should observe an increased K_m as the size of the surface potential is decreased, whereas a decreased K_m is the result for enzymes with a negatively charged substrate. The K_m for enzymes with a neutral substrate should be unaffected. The results for Jerusalem-artichoke mitochondria are summarized in Table 2, and there is an excellent agreement with the above predictions.

Effect of screening on $V_{\max}$ of membrane-bound enzymes

The $V_{\max}$ of single enzymes is normally not affected by changes in the surface potential (Wojtczak & Nalecz, 1979; Nalecz *et al.*, 1980). In

Fig. 5. Eadie-Hofstee plot of NADH oxidation by duroquinone in Jerusalem-artichoke mitochondria at low and high concentrations of cations

The medium contained 0.3M-sucrose, 5mM-Mops buffer, pH 7.2, 0.2 μ M-FCCP, 0.36 μ M-antimycin A, 0.18 mg of protein/ml and 10–140 μ M-NADH with (●) or without (○) 2mM-(DM)Br₂. The reaction was initiated by the addition of 0.3mM-duroquinone (final concentration). $V_{\max}$ is given in nmol of NADH/min per mg.

Table 2. *Effect of electrostatic screening on the kinetics of oxidation of substrates with different charge by Jerusalem-artichoke mitochondria*

High-cation conditions were obtained with 100 mM-KCl, except for NADH oxidation (both to O₂ and to duroquinone), where 2 mM-(DM)Br₂ was used. Results for the NADH-O₂ reaction (K_m and V_{max} under low-cation conditions 91 μ M and 395 nmol of NADH/min per mg respectively) are from Møller & Palmer (1981a); for the duroquinol-O₂ reaction they are the average for three independent preparations of mitochondria (including Fig. 3), for the cytochrome *c*-O₂ reaction for two preparations (including Fig. 4) and one preparation for the NADH-duroquinone reaction (Fig. 5).

Parameter	Part reaction	...	NADH-duroquinone	NADH-O ₂	Duroquinol-O ₂	Cytochrome <i>c</i> -O ₂
Charge of substrate at pH 7.2			Negative	Negative	Neutral	Positive
			Effect of cations (% change from value under low-cation conditions)			
Apparent K_m			-72%	-41%	-13%	+120%
V_{max}			+14%	+41%	+92%	+164%

contrast with this, the V_{max} for the multistep electron transfers from NADH (Møller & Palmer, 1981a) and duroquinol (Fig. 2 and Table 2) is strongly increased by electrostatic screening. This prompted us to suggest that diminished repulsion between different respiratory complexes and/or between complexes and charged lipids due to the lowered surface potential might result in an increased lateral mobility of the respiratory complexes and thus in an increased collision frequency and higher rates of oxidation (Møller & Palmer, 1981a; Palmer & Møller, 1982). From previous experiments we knew that the rate-limiting step(s) involved was probably located between ubiquinone and O₂ (Møller & Palmer, 1981a), in agreement with the results obtained by Hackenbrock and co-workers (Schneider *et al.*, 1980, 1982). The absence of an increase in V_{max} upon addition of cations for the NADH-duroquinone reaction (reaction 5 in Scheme 1; Fig. 5 and Table 2) is consistent with this view.

Cytochrome *c* oxidase constitutes one of the rate-limiting steps in the respiration of mammalian mitochondria (Groen *et al.*, 1982), although it does not appear to be stimulated by cations (see above). In contrast with this and with what is normally observed on single enzymes (see above), electrostatic screening strongly enhanced the V_{max} for cytochrome *c* oxidase in Jerusalem-artichoke mitochondria (Fig. 4 and Table 2). It would appear as if this enzyme might be responsible for part of the increased electron flow through the respiratory chain of plant mitochondria upon electrostatic screening. However, the stimulation of respiration by cations decreased substantially as longer segments of the respiratory chain were involved: cations only stimulated the duroquinol-O₂ reaction by one-half as much and the NADH-O₂ reaction by one-quarter as much as they stimulated the cytochrome *c*-O₂ reaction (Table 2). This

suggests that cytochrome oxidase is not rate-limiting when longer portions of the chain are involved and that there are other rate-limiting steps earlier in the chain, possibly involving ubiquinone, that are stimulated by electrostatic screening.

Other electron-transport systems

Electrostatic effects on thylakoid structure and function, including electron transport, are well documented (for reviews see Barber, 1980, 1982). However, plant membranes other than mitochondria and chloroplasts are charged and contain electron-transport chains/complexes. Thus antimycin A-insensitive NAD(P)H oxidation to cytochrome *c* can be observed with crude microsomal preparation and with purified plasma membranes (Lundborg *et al.*, 1981; Widell & Larsson, 1983). The negatively charged plasma membrane in plant roots (Gibrat & Grignon, 1982; Møller *et al.*, 1984; Körner *et al.*, 1984) is exposed to the variable ionic environment of the soil solution. It would be interesting to see to what extent the electron-transport properties of the root plasma membrane are affected by cation binding and electrostatic screening.

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References

- Barber, J. (1980) *Biochim. Biophys. Acta* **594**, 253–308
- Barber, J. (1982) *Annu. Rev. Plant Physiol.* **33**, 261–295
- Bomhoff, G. H. & Spencer, M. (1977) *Can. J. Biochem.* **55**, 1114–1117

- Chow, W. S. & Barber, J. (1980) *J. Biochem. Biophys. Methods* **3**, 173–185
- Coleman, J. O. D. & Palmer, J. M. (1971) *FEBS Lett.* **17**, 203–208
- Cowley, R. C. & Palmer, J. M. (1978) *Plant Sci. Lett.* **11**, 345–350
- Douce, R., Mannella, C. A. & Bonner, W. D., Jr. (1973) *Biochim. Biophys. Acta* **292**, 105–116
- Douzou, P. & Maurel, P. (1976) *C. R. Hebd. Séances Acad. Sci. Sér. D* **282**, 2107–2110
- Gibrat, R. & Grignon, C. (1982) *Biochim. Biophys. Acta* **692**, 462–468
- Groen, A. K., Wanders, R. J. A., Westerhoff, H. V., van der Meer, R. & Tager, J. M. (1982) *J. Biol. Chem.* **257**, 2754–2757
- Hackett, D. P. (1961) *Plant Physiol.* **36**, 445–452
- Henry, M. F. & Nyns, E. J. (1975) *Subcell. Biochem.* **4**, 1–65
- Johnston, S. P., Møller, I. M. & Palmer, J. M. (1979) *FEBS Lett.* **108**, 28–32
- Körner, L., Møller, I. M., Kjellbom, P. & Larsson, C. (1984) *Abstr. Congr. Fed. Eur. Soc. Plant Physiol.* **4th** 514–515
- Lawford, H. G. & Garland, P. B. (1972) *Biochem. J.* **130**, 1029–1044
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Lundborg, T., Widell, S. & Larsson, C. (1981) *Physiol. Plant.* **52**, 89–95
- Maurel, P. & Douzou, P. (1976) *J. Mol. Biol.* **102**, 253–264
- Møller, I. M. & Palmer, J. M. (1981a) *Biochem. J.* **195**, 583–588
- Møller, I. M. & Palmer, J. M. (1981b) *Physiol. Plant.* **53**, 413–420
- Møller, I. M. & Palmer, J. M. (1982) *Physiol. Plant.* **54**, 267–274
- 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
- Møller, I. M., Schwitzguébel, J.-P. & Palmer, J. M. (1982) *Eur. J. Biochem.* **123**, 81–88
- Møller, I. M., Palmer, J. M. & Johnston, S. P. (1983) *Biochim. Biophys. Acta* **735**, 289–297
- Møller, I. M., Lundborg, T. & Bérczi, A. (1984) *FEBS Lett.* **167**, 181–185
- Nalecz, M. J., Zborowski, J., Famulski, K. S. & Wojtczak, L. (1980) *Eur. J. Biochem.* **112**, 75–80
- Palmer, J. M. & Møller, I. M. (1982) *Trends Biochem. Sci.* **7**, 258–261
- Rich, P. R. & Bendall, D. S. (1980) *Biochim. Biophys. Acta* **592**, 506–518
- Rich, P. R., Wiegand, N. K., Blum, H., Moore, A. L. & Bonner, W. D., Jr. (1978) *Biochim. Biophys. Acta* **525**, 325–337
- Schneider, H., Lemasters, J. J., Hochli, M. & Hackenbrock, C. R. (1980) *J. Biol. Chem.* **255**, 3748–3756
- Schneider, H., Lemasters, J. J. & Hackenbrock, C. R. (1982) *J. Biol. Chem.* **257**, 10789–10793
- Smith, H. T., Ahmed, A. J. & Millett, F. (1981) *J. Biol. Chem.* **256**, 4984–4990
- Storey, B. T. (1976) *Plant Physiol.* **58**, 521–525
- Widell, S. & Larsson, C. (1983) *Physiol. Plant.* **57**, 196–202
- Wojtczak, L. & Nalecz, M. J. (1979) *Eur. J. Biochem.* **94**, 99–107
- Wojtczak, L., Famulski, K. S., Nalecz, M. J. & Zborowski, J. (1982) *FEBS Lett.* **139**, 221–224