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Nicotinamide nucleotide transhydrogenase from *Rhodobacter capsulatus*; the H^+/H^- ratio and the activation state of the enzyme during reduction of acetyl pyridine adenine dinucleotide

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Chromatophores from *Rhodobacter capsulatus* were incubated in the dark with NADPH and acetylpyridineadenine dinucleotide ($AcPdAD^+$) in the presence of different concentrations of myxothiazol. The transhydrogenase activity was monitored until an appropriate mass action ratio, $[AcPdAD^+][NADPH]/[AcPdADH][NADP^+]$, was reached. The sample was then illuminated and the initial rate of either $AcPdAD^+$ reduction by NADPH or AcPdADH oxidation by $NADP^+$ was recorded. The ratio of H^+ translocated per H^- equivalent transferred by transhydrogenase was calculated from the value of the membrane potential ($\Delta pH = 0$) at which illumination caused no net reaction in either direction. The mean value for the H^+/H^- ratio was 0.55. At greater values of $[AcPdAD^+][NADPH]/[AcPdADH][NADP^+]$ than were employed in the above experiments and over a wider range of concentrations of myxothiazol, it was found that incremental increases in the membrane potential always gave rise to a decrease, never an increase in the rate of $AcPdAD^+$ reduction. In contrast to the H^+-ATP synthase, there is no evidence of any activation/deactivation of H^+-transhydrogenase by the protonmotive force.

Introduction

H^+-Thase, found in the inner membranes of mitochondria and the cytoplasmic membranes of many bacteria, catalyses the reduction of $NADP^+$ by NADH. The reaction, which is reversible, is coupled to the flux of protons across the membrane:

$$NADH + NADP^+ + nH^+_{out} \rightleftharpoons NAD^+ + NADPH + nH^+_{in} \quad (1)$$

where H^+_{out} and H^+_{in} indicate that in the normal physiological direction the reaction is driven by a protonmotive force (Δp) generated by respiratory or photosynthetic electron transport (see reviews [1–3]).

Central to our understanding of the coupling mechanism is an accurate measurement of the value of n the ratio of H^+ translocated to H^- transferred. There have been several earlier attempts in different laboratories to estimate the H^+/H^- ratio by both kinetic and equilibrium procedures [1,4–10] and a consensus has been reached that the value may be 1.0. In this paper we describe a new procedure for measuring the H^+/H^- ratio. It is based on experiments which determine precisely the conditions in which the H^+-Thase reaction and Δp are in equilibrium. We also re-examine the reasons for adopting a consensus value of 1.0 for the H^+/H^- ratio.

The experiments were performed with everted membrane vesicles, or chromatophores, from *Rhodobacter capsulatus* which have an active H^+-Thase [9,11]. In chromatophores, Δp is conveniently generated by the cyclic photosynthetic electron transport system. The electrical component ($\Delta\Psi$) of the protonmotive force can be measured by recording electrochromic absorbance changes of intrinsic carotenoid pigments [12] under conditions in which the chemical component (ΔpH) is zero.

Analogue substrates are commonly used to measure the reaction catalysed by H^+-Thase. The reduction of the NAD^+ analogue, $AcPdAD^+$, by NADPH, equivalent to the reverse of Eqn. (1) can be measured at around 375 nm. The method that has been developed here to measure the H^+/H^- ratio also relies on the fact that the standard redox potential of $AcPdAD^+$/AcPdADH is approx. 70 mV more positive than that of

Abbreviations: H^+-Thase, nicotinamide nucleotide H^+-transhydrogenase; $AcPdAD^+$, 3-acetylpyridineadenine dinucleotide; $\Delta\Psi$, membrane potential; ΔpH, transmembrane pH gradient.

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$NADP^+$/NADPH [13]. Thus, with the physiological substrates even only moderate values of Δp lead to large values of the equilibrium mass action ratio, $[NADPH][NAD^+]/[NADP^+][NADH]$, and consequently, errors in the measurement of the denominator are highly critical. However, when AcPdAD$^+$/AcPdADH replaces NAD^+/NADH the reaction at high $\Delta\Psi$ is more centrally poised and therefore the mass action ratio can be measured with greater accuracy.

These properties of the reaction, NADPH → AcPdAD$^+$, have also been exploited in the experiments described below to try to determine whether H^+-Thase can be "activated" by Δp. There have been suggestions that increases in Δp might shift the enzyme into a more active conformational state and that this could partly explain the effect of Δp on the rate of reaction in the forward direction [14,15]. There is very good evidence that another active consumer of Δp, the F_0F_1-ATPase, is controlled in this manner, although the mechanism of the enzyme activation is different in different organisms [16–18]. The question as to whether H^+-Thase is activated by Δp is addressed by examining the dependence of the rate of reduction of AcPdAD$^+$, when the chemical affinity of the reaction is very high, upon the value of Δp.

Methods

Rb. capsulatus strain 37b4 (from Dr. G. Drews, University of Freiburg) was grown and chromatophores were prepared (in a medium containing 10% sucrose, 30 mM NaCl, 2 mM $MgCl_2$, 50 mM Tricine-NaOH (pH 7.6)) and assayed for bacteriochlorophyll concentration as described [19].

The extinction cofficient of AcPdADH at 363 nm is given as $E = 9.12$ mM^{-1} cm^{-1} (pH 9.5) and for NADPH at 334 nm, $E = 6.178$ mM^{-1} cm^{-1} (pH 7.6) [13,20]. From these values and from the measured absorbance spectra at defined pH, we calculated the extinction coefficient at 375–455 nm for the reduction of AcPdAD$^+$ during simultaneous oxidation of NADPH to be 6.10 mM^{-1} cm^{-1} (pH 7.6).

Stock solutions of NADPH in 10 mM triethanolamine-HCl (pH 7.6) were assayed using the extinction coefficient given above and of AcPdAD$^+$ in 20 mM sodium phosphate (pH 7.5) using the extinction coefficient at 260 nm, $E = 16.22$ mM^{-1} cm^{-1} [13].

The $G^{\circ\prime}$ for the reaction

$$AcPdADH + NADP^+ \rightleftharpoons AcPdAD^+ + NADPH$$

was calculated from standard redox potentials for AcPdAD$^+$/AcPdADH and $NADP^+$/NADPH of −248 mV and −320 mV, respectively [13].

Assays of AcPdAD$^+$ reduction by NADPH at 375–455 nm were carried out at 30° in a medium containing 10% sucrose, 30 mM KCl, 2 mM $MgCl_2$, 50 mM Tricine-KOH, (pH 7.6), 1.0 μg ml^{-1} rotenone, 0.2 μg ml^{-1} venturicidin, 1.0 μg ml^{-1} nigericin, chromatophores to 10 μM bacteriochlorophyll and myxothiazol and nucleotide concentrations given in the figure legends in a Shimadzu UV-3000 dual wavelength spectrophotometer fitted with a high-power GaAlAs emitter to provide actinic illumination [21]. Electrochromic absorbance changes of carotenoids were measured under the same conditions but at 528–511 nm. The electrochromic absorbance changes were calibrated in a medium containing 10% sucrose, 30 mM NaCl, 2 mM $MgCl_2$, 50 mM Tricine-NaOH (pH 7.6) by applying K^+-diffusion potentials [22].

All nucleotides were purchased from Sigma.

Results

The H^+/H^- ratio of H^+-Thase in chromatophores of Rb. capsulatus

The experimental procedure is demonstrated in Fig. 1. Traces *a* and *b* show the time-course for the reduc-

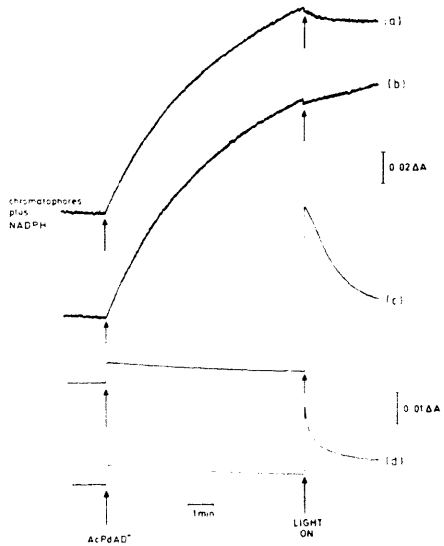

Fig. 1. The kinetics of H^- transfer between AcPdADH and NADPH and of $\Delta\Psi$ formation in suspensions of chromatophores from *Rb. capsulatus*. See Methods. Traces (a) and (b) represent the reduction of AcPdAD$^+$ (upward direction) and traces (c) and (d), the formation of $\Delta\Psi$. In experiments (a) and (c) the myxothiazol concentration was 10 nM and in experiments (b) and (d) it was 40 nM. Separate experiments in the absence of nucleotides revealed that the small, rapid absorbance decreases at the onset of illumination in traces (a) and (b) were due to background changes in the chromatophore pigments (not shown).

tion of AcPdAD$^+$ by NADPH by a dark suspension of chromatophores of *Rb. capsulatus*. From an accurate measurement of the starting concentrations of nucleotides and from the relevant extinction coefficient for AcPdAD$^+$ reduction during NADPH oxidation (see Methods), the mass action ratio, [AcPdAD$^+$]·[NADPH]/[AcPdADH][NADP$^+$], can be calculated throughout the course of the reaction. After an appropriate period of time the photosynthetic light was switched on. The method relies on the assumption that, if the value of Δp generated by photosynthetic electron flow is large then the direction of the transhydrogenase reaction will reverse (from NADPH → AcPdAD$^+$ to AcPdADH → NADP$^+$) at the onset of illumination – see traces *a* and *c*. If, on the other hand, Δp is small, then the transhydrogenase reaction will continue in the direction NADPH → AcPdAD$^+$ – see traces *b* and *d*. If the free energy available to the H$^+$-Thase from the transfer of nH$^+$ by the protonmotive force ($\Delta G_{CHEMIOS}$) is balanced by the chemical affinity for hydride transfer (ΔG_{THASE}), then there will be no net reaction.

$$\Delta G_{CHEMIOS} = -nF\Delta p$$

$$\Delta G_{THASE} = \Delta G^{\circ\prime} + RT \ln[\text{AcPdAD}^+][\text{NADPH}] / [\text{AcPdADH}][\text{NADP}^+]$$

Hence,

$$\Delta G^{\circ\prime} + RT \ln[\text{AcPdAD}^+][\text{NADPH}]/[\text{AcPdADH}][\text{NADP}^+] = -nF\Delta p$$

from which, given Δp, n can be determined. Thus, in a series of experiments similar to those shown in Fig. 1, chromatophores were treated with different concentrations of myxothiazol specifically to inhibit the cytochrome bc_1 complex to limit the value of Δp generated by photosynthetic electron transport (myxothiazol had no direct effect on H$^+$-Thase, as evidenced by the kinetics of AcPdAD$^+$ reduction in the dark). For each concentration of myxothiazol, the initial rate of the transhydrogenase reaction and the value of $\Delta\Psi$ shortly after the onset of illumination were recorded. The results are plotted in Fig 2. Positive values of the transhydrogenase rate represent the reaction in the direction AcPdADH → NADP$^+$ and negative values represent the reaction in the direction NADPH → AcPdAD$^+$ Three different mass action ratios were chosen by allowing the H$^+$-Thase reaction to proceed in the dark for 5, 8 or 10 min before switching on the light. The value of n was calculated from the three sets of data using the null points ($\Delta G = 0$) estimated from Fig. 2.

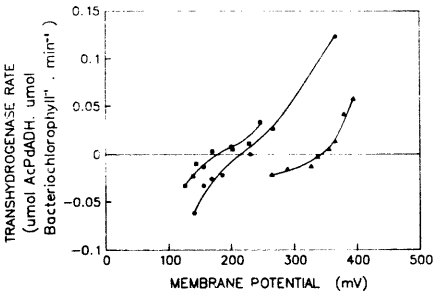

Fig. 2. The H$^+$/H$^-$ ratio of H$^+$-Thase in chromatophores from *Rb. capsulatus*. Experiments were performed as in Fig. 1. Positive values of the transhydrogenase rate represent the reaction in the direction AcPdADH → NADP$^+$ and negative values represent the reaction in the direction NADPH → AcPdAD$^+$. ▲, the dark preincubation (in the presence of nucleotides before illuminating) was for 5 min to reach a mass action ratio of 1.14; ●, the dark preincubation period was for 8 min to reach a mass action ratio of 4.86; ■, the dark preincubation time was for 10 min to reach a mass action ratio of 8.33. The concentration of myxothiazol was varied between 0 and 48 nM to achieve the given values of membrane potential during illumination

There are several points to note in the context of the experiments shown in Fig. 1 and the compilation of data shown in Fig. 2.

(a) All experiments were performed in the presence of nigericin to eliminate any contribution from ΔpH and to ensure that $\Delta\Psi$ was the sole contributor to Δp.

(b) The reference wavelength used in the measurement of AcPdAD$^+$ was fine-tuned to 455 nm in the experiments shown in order to minimise any background absorbance changes arising from chromatophore pigments at the onset of illumination. The most suitable reference, which was determined in control experiments in the absence of nucleotides, varied slightly amongst different preparations.

(c) The addition of AcPdAD$^+$ and NADPH to the dark suspension at the start of the experiment (Fig. 1, traces *c* and *d*), led to an increase in $\Delta\Psi$ due to the inward translocation of H$^+$ by transhydrogenase, as predicted by Eqn. 1 and see Ref. 23. The values of $\Delta\Psi$ used in Fig. 2 are all expressed relative to a zero value established by addition of 1.0 μM carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone at the end of the experiment.

(d) During prolonged illumination $\Delta\Psi$ slowly subsided to a lower value (Fig. 1, Traces *c* and *d*), a consequence of poor redox poising in the system in the absence of added reductant. This permitted the H$^+$-Thase reaction to revert gradually towards increased rates in the direction of AcPdAD$^+$ reduction (Fig. 1, Traces *a* and *b*). Because of this, and because it was necessary to compile sets of data at the same mass

action ratio, it was essential to record the initial rates at the onset of illumination. As is evident from the figure, a period of approx. 15 s was required for an accurate measurement of these rates and during such a period $\Delta\Psi$ declined in value, typically by approx. 10%. The mean value of $\Delta\Psi$ after 2 s and 10 s of excitation was taken in the construction of Fig. 2; any error incurred by this procedure was less than the range of scatter on the data points in the figure.

H^+-Thase does not appear to undergo activation by $\Delta\Psi$

Experiments similar to those shown in Fig. 1 were performed with a shorter period of incubation in the dark in the presence of nucleotides and therefore at a larger mass action ratio at the point of illumination. The object was to ensure that at the onset of illumination the chemical affinity of the reaction was greatly in favour of AcPdAD$^+$ reduction by NADPH (ΔG ranged from −31 800 to −14 800 joule deg^{-1} mol^{-1} in the experiments of Fig. 3). Moreover, the experiments were carried out at higher concentrations of myxothiazol to restrict more heavily the value of $\Delta\Psi$ generated during illumination. The rationale was that under these conditions the thermodynamic tendency – the 'driving force' effect of $\Delta\Psi$ on the reaction – was minimised and the likelihood of revealing conformational activation of the enzyme was increased.

Fig. 3 shows the dependence of the initial rate of reduction of AcPdAD$^+$ at the onset of illumination upon the value of $\Delta\Psi$. The same convention as in Fig. 2 is adopted, that is the rates of reduction of AcPdAD$^+$ by NADPH are assigned negative values. The essential observation is that over the entire range of $\Delta\Psi$ values an increase in $\Delta\Psi$ was always accompanied by a decrease, never an increase, in the rate of reduction of AcPdAD$^+$ by NADPH.

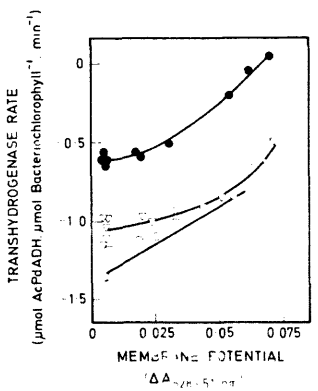

Fig. 3. The dependence of the rate of AcPdAD$^+$ reduction with NADPH by H^+-Thase from *Rb. capsulatus* on $\Delta\Psi$. Experiments were performed as in Figs. 1 and 2. ○, the dark preincubation time was 10 s; ▽, for 1 min; ●, for 5 min. The concentration of myxothiazol was varied between 0 and 200 nM to achieve the given values of membrane potential during illumination. In contrast to Fig. 2, the electrochromic absorbance changes of this preparation of chromatophores were not calibrated.

Discussion

The procedure that we have used above to estimate the H^+/H^- ratio relies on precise determination of the equilibrium position between the H^+-Thase reaction and the protonmotive force. By approaching the point at which the rate of AcPdAD$^+$ reduction by NADPH is equal to the rate of AcPdADH oxidation by NADP$^+$ from both high and low values of Δp, the tendency of the reaction to proceed is determined. At the null point, $\Delta G = 0$ and the equilibrium assumption should be justified. The actual rates of the transhydrogenase reaction are not critical, only whether they are positive or negative. By clustering the experiments close to the null point, an accurate indication of the equilibrium position was obtained. Since the membrane potential is measured directly the value calculated for the H^+/H^- ratio is independent of the coupled state of the membrane.

This method is complementary to the more conventional equilibrium procedure in which the nucleotide substrates are incubated with energised membranes and periodically assayed until their concentrations become constant [10,24]. In those experiments the criterion for attainment of equilibrium is that similar mass action ratios are reached from both high and low starting values. However, a significant problem with that method is that it proves to be difficult to eliminate the NADH oxidase activity of chromatophore membranes completely, even in the presence of high concentrations of rotenone. With the physiological substrates the problem is especially acute since small errors in the value of NAD$^+$ are critical in the calculation of n. An advantage of the present method is that the calculation procedure partly compensates for the influence of residual NADH oxidase activity on the mass action ratio. It does still suffer from the other disadvantage mentioned [10], that a population of chromatophores with uncoupled H^+-Thase would lead to an underestimate of the value of n.

The H^+/H^- ratios for the three different sets of experiments (three different mass action ratios) shown in Fig. 2 are 0.64, 0.58 and 0.44, with a mean of 0.55. This value is compared in Table I with others taken from the literature. The methods to determine H^+/H^- ratios for chromatophores of *Rb. capsulatus* have all used electrochromic absorbance changes as a measure of $\Delta\Psi$. The electrochromic technique has proved reliable in a wide range of experiments and it is important

TABLE I

H^+/H^- ratios for trans hydrogenase

Biological material and reference	H^+/H^- ratio
Mitochondria	
Earle and Fisher, 1980 [4]	0.84 (extrapolating to 1.0)
Wu et al., 1981 [5]	0.73, 0.64
Anderson et al., 1981, [6]	0.6
Wu et al., 1986 [7]	0.35–0.9
Hoek and Rydstrom, 1988 [1]	0.73–0.89 [a]
Eytan et al., 1987 [8]	1.0
Chromatophores from *Rb. capsulatus*	
Cotton et al., 1989 [9]	0.4
Jackson et al., 1990 [10]	0.72
Palmer and Jackson, this paper	0.55

[a] Calculated from the range of Δp values normally measured in submitochondrial particles.

to note that two unrelated calibration procedures involving fundamentally different measurements have been employed in the methods to estimate the H^+/H^- ratios. In the present work and in Ref. 10, the electrochromic absorbance changes were calibrated using K^+ diffusion potentials, whereas in Ref. 9 ionic currents measured by electrochromism were calibrated from an estimate of the amount of charge translocated across the chromatophore membranes during a single turnover light flash. Even so, it should be appreciated that error in the estimation of $\Delta\Psi$ will be directly reflected in calculation of the value of n. The H^+/H^- ratios for mitochondrial H^+-Thase from Fisher's group [4–7] in Table I were all determined in liposomes by comparison of the rate of H^+ uptake with the rate of either $AcPdAD^+$ reduction or NAD^+ reduction by NADPH. The measured values were less than those shown in the table but, because initial rates were not measured, an extrapolation to zero time was required to estimate the 'true' H^+/H^- ratio under conditions of negligable proton efflux. However, the precise extrapolation is difficult to justify. The values were also inflated by subtraction of valinomycin-independent transhydrogenase activity, which was assumed to originate from non-incorporated enzyme – see Ref. 3. Table I includes a value of the H^+/H^+ ratio – see Ref. 1 – calculated on the basis of the equilibrium mass action ratio for H^+-Thase in submitochondrial particles [24] and the range of values of Δp that are normally measured in this system (see Ref. 25). The H^+/H^- ratio of 1.0 by Eytan et al. [8] was calculated from the ratio of the rates of transhydrogenase and of ATP hydrolysis, assuming that $H^+/ATP = 3$ and that there is no proton leak in co-reconstituted vesicles. Whether or not these assumptions can be justified remains to be established.

Observations on the inhibitory effects of dicyclohexylcarbodiimide on H^- transfer and H^+ translocation by mitochondrial H^+-Thase were taken to indicate that the enzyme may 'slip' [26,27], but these results were later called into question [28,29]. There is no obvious trend in the values summarised in Table I (e.g., low H^+/H^- ratios at high Δp) to suggest that the spread of the data results from slip. Thus, it has generally been assumed that the H^+/H^- ratio should be an integer; 1.0 would be the most likely stoichiometry on the basis of the values shown in Table I. The errors and uncertainties involved in the methods used to date are such that a value of 1.0 is not inconceivable However, the mean value of the H^+/H^- ratios shown in Table I is closer to 0.5 and consideration ought to be given to the possibility that this is the actual value. Plausible coupling mechanisms in which H^+ translocation is directly involved in the chemistry of the H^- transfer reaction ('direct' coupling – see Ref. 3) demand an H^+/H^- stoichiometry of 1.0 or greater. 'Indirect' coupling mechanisms in which conformational changes link the H^+ translocation reaction with H^- transfer have hitherto invoked (or assumed) a stoichiometry of 1.0 [30–32]. An H^+/H^- ratio of 0.5 would impose major constraints on the nature of the energy coupling reaction but one way in which this value could be accommodated takes into account the fact that the H^+-Thases of both mitochondria and of *E. coli* seem to function as dimers [33–35]. It may be envisaged that H^+ conduction through a single channel in the dimer is conformationally coupled to both catalytic sites in the monomers. This view is consistent with the observations that mitochondrial H^+-Thase displays 'half of the sites reactivity' with respect to some covalent inhibitors [36,37].

The results of the experiments shown in Fig. 3 show no evidence of any conformational activation of H^+-Thase in *Rb. capsulatus*. When conditions were chosen greatly to favour the reverse H^- transfer reaction (from NADPH to $AcPdAD^+$) even up to substantial values of Δp, there was never any increase in the rate of reaction for an incremental increase in Δp. It unlikely on this basis that H^+-Thase exists in a low-activity form at low Δp and in a high-activity form at high Δp, i.e., the enzyme is only controlled by Δp in the sense that Δp provides the driving force for the reaction. To date, we have not found any significant modifying effect on the H^+-Thase activity (in the forward direction) in chromatophore membranes by a number of metabolites. H^+-Thase from *Rb. capsulatus* is, however, subject to pronounced product inhibition Ref. 38 and see Ref. 1. It is conceivable that under physiological conditions in the cell, H^+-Thase is subject to the thermodynamic control of the nucleotide mass action ratio and the value of Δp, and to kinetic control only by the concentrations of the reactants and products.

Acknowledgements

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References

1 Rydstrom, J. and Hoek, J.B. (1988) Biochem. J. 254, 1–10.
2 Rydstrom, J., Persson, B. and Carlenor, E. (1987) in Pyridine nucleotide coenzymes: chemical, biochemical and medical aspects, Vol. 2B (Dolphin, D., Poulson, R. and Avramovic, O., eds), pp. 433–460, John Wiley and Sons, New York.
3 Jackson, J.B. (1991) J. Bioenerg. 23, 715–741.
4 Earle, S.R. and Fisher, R.R. (1980) J. Biol. Chem. 255, 827–830.
5 Wu, L.N.Y., Earle, S.R. and Fisher, R.R. (1981) J. Biol. Chem. 256, 7401–7408.
6 Anderson, W.M., Fowler, W.T., Pennington, R.M. and Fisher, R.R. (1981) J. Biol. Chem. 256, 1888–1895.
7 Wu, L.N.Y., Alberta, J.A. and Fisher, R.R. (1986) Methods Enzymol. 126, 353–360.
8 Eytan, G.D., Persson, B., Ekebacke, A. and Rydstrom, J. (1987) J. Biol. Chem. 262, 5008–5014.
9 Cotton, N.P.J., Lever, T.M., Nore, B.F., Jones, M.R. and Jackson, J.B. (1989) Eur. J. Biochem. 182, 593–603.
10 Jackson, J.B., Cotton, N.P.J., Lever, T.M., Cunningham, I.J., Palmer, T. and Jones, M.R. (1990) in Molecular biology of membrane-bound complexes in phototrophic bacteria (Drews, G. and Dawes, E.A., eds.), pp. 415–124, Plenum, New York.
11 Cotton, N.P.J., Myatt, J.F. and Jackson, J.B. (1987) FEBS Letts. 219, 88–92.
12 Jackson, J.B. (1988) in Bacterial Energy Transduction (Anthony, C., ed.), pp. 317–375, Academic Press, London.
13 Woenckhaus, C. and Jeck, R. (1987) in Pyridine nucleotide coenzymes: chemical, biochemical and medical aspects, Part A (Dolphin, D., Poulson, R. and Avramovich, O., eds.), pp. 449–568, John Wiley & Sons, New York.
14 Rydstrom, J. (1977) Biochim. Biophys. Acta 463, 155–184.
15 Galante, Y.M., Lee, Y. and Hatefi, Y. (1980) J. Biol. Chem. 255, 9641–9646.
16 Pederson, P.L., Schwerzmann, K. and Cintron, N. (1981) Curr. Top. Bioenerg. 11, 149–199.
17 Klionsky, D.J., Brusilow, W.S.A. and Simoni, R.D. (1984) J. Bacteriol. 160, 1055–1060.
18 Ketcham, S.R., Davenport, J.W., Warncke, K. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7286–7293.
19 Clark, A.J., Cotton, N.P.J. and Jackson, J.B. (1983) Biochim. Biophys. Acta 723, 440–453.
20 Rizzo, V., Pande, A. and Luisi, P.L.(1987) in Pyridine nucleotide coenzymes chemical, biochemical and medical aspects, Part A (Dolphin, D., Poulson, R. and Avramovic, O., eds.), pp. 99–161, John Wiley & Sons, New York.
21 Palmer, T. and Jackson, J.B. (1990) FEBS Lett. 277, 45–48.
22 Jackson, J.B. and Clark, A.J. (1981) in Vectorial reactions in electron and ion transport in mitochondria and bacteria (Palmieri, F., ed.), pp. 371–379, Elsevier, Amsterdam.
23 Oustroumov, S.A., Samuilov, V.D. and Skulachev, V.P. (1973) FEBS Lett. 31, 27–30.
24 Lee, C.-P. and Ernster, L. (1964) Biochim. Biophys. Acta 81, 187–190.
25 Ferguson, S.J. and Sorgato, M.C. (1982) Annu. Rev. Biochem. 51, 185–217.
26 Pennington, R.M. and Fisher, R.R. (1981) J. Biol. Chem. 256, 8963–8969.
27 Persson, B., Enander, K., Tang, H.L. and Rydstrom, J. (1984) J. Biol. Chem. 259, 8626–8632.
28 Phelps, D.C. and Hatefi, Y. (1984) Biochemistry 23, 6340–6344.
29 Wakabayashi, S. and Hatefi, Y. (1987) Biochem. Int. 15, 915–924.
30 Yamaguchi, M. and Hatefi, Y. (1989) Biochemistry 28, 6050–6056.
31 Fisher, R.R. and Earle, S.R. (1982) in The pyridine nucleotide coenzymes (Everse, J., Andersson, B. and You, K.-S., eds.), pp. 279–324, Academic Press, New York.
32 Rydstrom, J., Lee, C.-P. and Ernster, L.(1981) in Chemiosmotic proton circuits in biological membranes (Skulachev, V.P. and Hinkle, P.C., eds.), pp. 483–508, Addison-Wesley, Reading, MA.
33 Wu, L.N.Y. and Fisher, R.R. (1983) J. Biol. Chem. 258, 7847–7851.
34 Persson, B., Ahnstrom, G. and Rydstrom, J. (1987) Arch. Biochem. Biophys. 259, 341–349.
35 Hou, C., Potier, M. and Bragg, P.D. (1990) Biochim. Biophys. Acta 1018, 61–66.
36 Phelps, D.C. and Hatefi, Y. (1984) Biochemistry 23, 4475–4480.
37 Phelps, D.C. and Hatefi, Y. (1985) Biochemistry 24, 3503–3507.
38 Lever, T.M., Palmer, T., Cunningham, I.J., Cotton, N.P.J. and Jackson, J.B. (1991) Eur. J. Biochem. 197, 247–245.