

# The dependence of the rate of transhydrogenase on the value of the protonmotive force in chromatophores from photosynthetic bacteria

N.P.J. Cotton, J.F. Myatt and J.B. Jackson

*Department of Biochemistry, University of Birmingham, PO Box 363, Birmingham B15 2TT, England*

Received 26 March 1987; revised version received 28 April 1987

In conditions where the pH gradient is negligible, the rate of the pyridine nucleotide transhydrogenase in chromatophores of *Rhodobacter capsulatus* has a threshold dependence on membrane potential. The relationship is similar when either antimycin or myxothiazol or carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone is used to depress the membrane potential. The relationship is distorted when membrane potential is reduced by lowering the photosynthetic light intensity.

Transhydrogenase; Protonmotive force; Chromatophore; Photosynthetic bacteria; Chemiosmotic hypothesis;

## 1. INTRODUCTION

The precise dependence of the rate of ATP synthesis on the value of protonmotive force ( $\Delta p$ ) in chloroplasts, mitochondria and bacteria has been controversial. Some observations suggest that the relationship is unique [1–4], others that it is influenced by the agent used to lower  $\Delta p$  [5–9]. The latter result calls for refinements of the chemiosmotic hypothesis of energy coupling between electron transport and ATP synthesis (e.g. [10]). It has been argued and counter-argued that

systematic experimental errors might distort the true relationship (see [1,4,6]). Alternatively different biological systems might behave in different ways – some showing a greater, others showing a lesser tendency towards delocalisation of the high-energy intermediate [6].

The experiments of Melandri and colleagues [8,9] on chromatophores from photosynthetic bacteria were amongst the first to show discrepancies. These workers found that the dependence of the rate of ATP synthesis on  $\Delta p$  was steeper when antimycin was used as the titrant than when uncoupling agent was used. Electrochromic absorbance changes and 9-aminoacridine fluorescence quenching were used to measure  $\Delta p$ , and ATP was assayed in acid-quenched samples [8,9].

Transhydrogenase which catalyses the transfer of hydride ion equivalents from NADH to NADP<sup>+</sup> is another enzyme which is driven by the free enthalpy released during electron transport in mitochondria and bacteria [11]. Chromatophores are capable of high rates of light-driven transhydrogenase [12]. Because thio-NADP<sup>+</sup> is a good

Correspondence address: J.B. Jackson, Department of Biochemistry, University of Birmingham, PO Box 363, Birmingham B15 2TT, England

**Abbreviations:** FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone;  $\Delta\psi$ , bulk phase electric potential difference across the chromatophore membrane;  $\Delta pH$ , bulk phase pH gradient across the chromatophore membrane;  $\Delta p$ , protonmotive force;  $J_t$ , rate of the pyridine nucleotide transhydrogenase

substrate for the enzyme the activity can be measured in continuous assay with excellent precision by making use of the difference in the wavelength of maximum absorbance of NADH and thio-NADPH [13]. This presents the opportunity to determine the dependence of the rate of transhydrogenase ( $J_t$ ) upon the value of  $\Delta p$  by spectrophotometric assays on virtually identical samples thus minimising systematic errors.

## 2. MATERIALS AND METHODS

*Rhodobacter capsulatus* strain N22 (formerly *Rhodopseudomonas capsulata*) was grown and chromatophores were prepared as described [14,15]. Transhydrogenase rates were assayed in a medium containing, in 10% sucrose, 100 mM KCl, 1 mM  $MgCl_2$ , 50 mM Tricine, pH 7.6, 66  $\mu M$  thio-NADP<sup>+</sup>, 133  $\mu M$  NADH, 1  $\mu g/ml$  nigericin, 1  $\mu g/ml$  rotenone, 0.2  $\mu g/ml$  venturicidin and chromatophores to give a final bacteriochlorophyll concentration of 10  $\mu M$ . The production of thio-NADPH was recorded in a sensitive single-beam spectrophotometer at 395 nm ( $\epsilon_{mM} = 11.3 \text{ cm}^{-1}$ ) where the absorbance contribution from NADH is negligible [13]. Each sample was pre-incubated, aerobically, in the dark for 10 min at 30°C before illumination. Photosynthetic illumination was provided at 90° to the measuring beam by a 250 W quartz halogen lamp filtered through one layer of Wratten 88A filter. Where shown, the light intensity was reduced using calibrated neutral density filters. The nucleotide substrate concentrations were considerably in excess of their  $K_m$  values and did not decrease by more than 5% during the course of the assay. A fresh sample was used for each recording. The value of  $\Delta\psi$  was estimated from light-induced electrochromic absorbance changes of the endogenous carotenoid pigments at 503 nm in parallel experiments under similar conditions except that the thio-NADPH was replaced by NADP<sup>+</sup>. A series of experiments showed that this did not influence the measured  $\Delta\psi$  within the precision of the experiment ( $\pm 3\%$ ). The true baseline of the electrochromic absorbance change was established by the addition of 1  $\mu M$  FCCP to darkened chromatophore suspensions. The electrochromic absorbance change was calibrated by applying  $K^+$ -diffusion potentials in the presence of valinomycin [16].

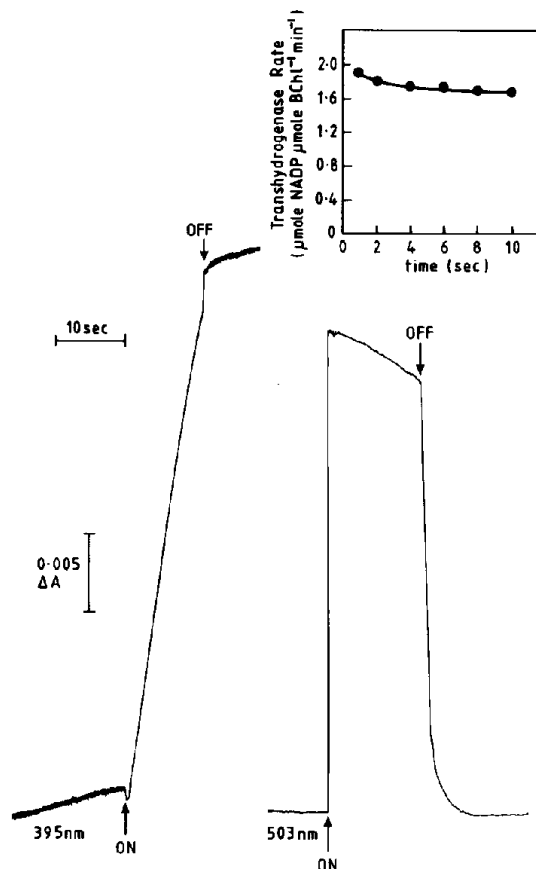


Fig.1. Recordings of transhydrogenase and electrochromic absorbance changes. See section 2. The inset shows how the rate of transhydrogenase decreases with the time of illumination.

## 3. RESULTS

In preliminary experiments suitable conditions for the measurement of  $J_t$  in *Rb. capsulatus* chromatophores were established. Venturicidin was found to stimulate transhydrogenase activity by approx. 55%, presumably by blocking energy dissipation through the ATP synthase. Nigericin was routinely added to eliminate the contribution of the transmembrane pH gradient to  $\Delta p$  and rotenone was used to block the chromatophore NADH dehydrogenase. Fig.1 shows representative recordings of  $J_t$  and of the electrochromic absorbance change in the standard conditions. Signal averaging was unnecessary on the time scale employed.

In the dark period before illumination there was a low but measurable transhydrogenase rate. This was stimulated approx. 20-fold by light. On a bacteriochlorophyll basis transhydrogenase had a rate comparable with that of chromatophore ATP synthesis. The rate of transhydrogenase and the value of  $\Delta\psi$  declined slightly during illumination (see inset to fig.1). In subsequent experiments these parameters were recorded after 10 s of illumination.

The data shown in figs 2 and 3 summarise the results of experiments in which  $\Delta\psi$  was depressed in titrations with antimycin A, an inhibitor of the quinone reductase site in the cytochrome  $b/c_1$  complex, myxothiazol, an inhibitor of the quinol oxidase site in the  $b/c_1$  complex and with the uncoupling agent, FCCP. Fig.2 demonstrates that the dependence of  $J_t$  on  $\Delta\psi$  was identical whether FCCP or antimycin was used as the titrant. Note that in contrast to results in a recent publication from our laboratory [17], antimycin A at moderate concentrations ( $0.2 \mu\text{M}$ ) strongly inhibited the

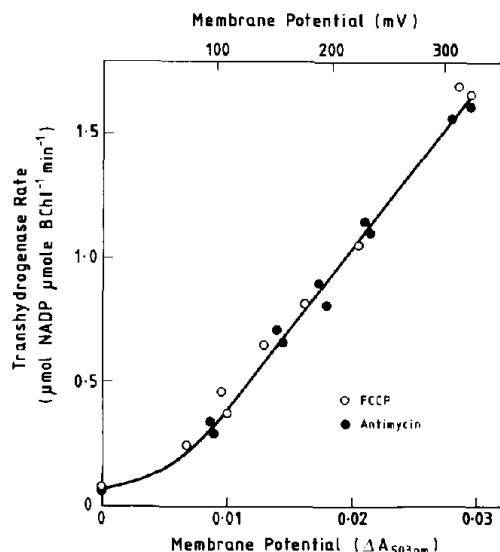


Fig.2. The dependence of transhydrogenase rate on  $\Delta\psi$  revealed by titrations with FCCP and antimycin A. From a series of experiments similar to that shown in fig.1. The values of  $\Delta\psi$  and the rates of transhydrogenase were estimated after 10 s illumination. Approx.  $0.08 \mu\text{M}$  FCCP and  $0.03 \mu\text{M}$  antimycin A were required for 50% inhibition of the transhydrogenase rate.

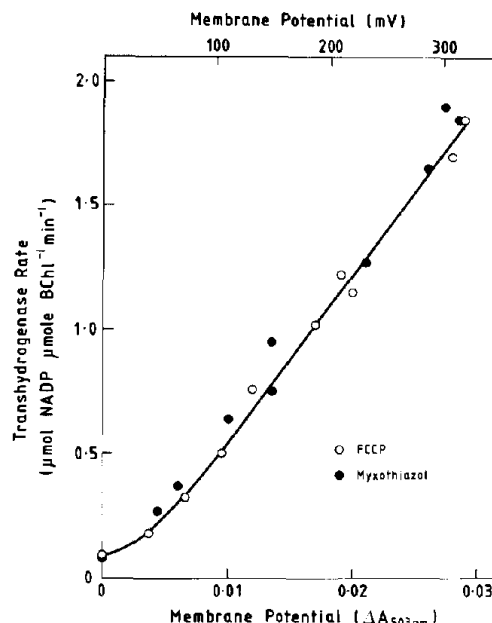


Fig.3. The dependence of transhydrogenase rate on  $\Delta\psi$  revealed by titrations with FCCP and myxothiazol. Approx.  $0.08 \mu\text{M}$  FCCP and  $0.06 \mu\text{M}$  myxothiazol were required for 50% inhibition of the transhydrogenase rate.

light-induced  $\Delta\psi$ . This is because the experiments reported here were carried out in the absence of succinate and in the presence of nigericin which has a very slight protonophoric activity. This becomes significant at low value of  $\Delta\psi$  because of the intrinsic non-ohmic conductance properties of the chromatophore membrane [17]. A similar result is shown in fig.3 on a different chromatophore preparation and using FCCP and myxothiazol. The relation between  $J_t$  and  $\Delta\psi$  was the same, independently of how  $\Delta\psi$  was varied.

There was a threshold in the region of 50 mV for the transhydrogenase rate although even at  $\Delta\psi = 0$  there was a low but significant activity. Above the threshold, the increase in  $J_t$  with  $\Delta\psi$  was approximately linear up to approx. 300 mV although in some chromatophore preparations there was an indication of rate saturation at high  $\Delta\psi$  (see the FCCP titration in fig.4).

A different profile for the dependence of  $J_t$  on  $\Delta\psi$  was observed when  $\Delta\psi$  was lowered by reducing the photosynthetic light intensity. Fig.4 shows the results of two sets of experiments to compare

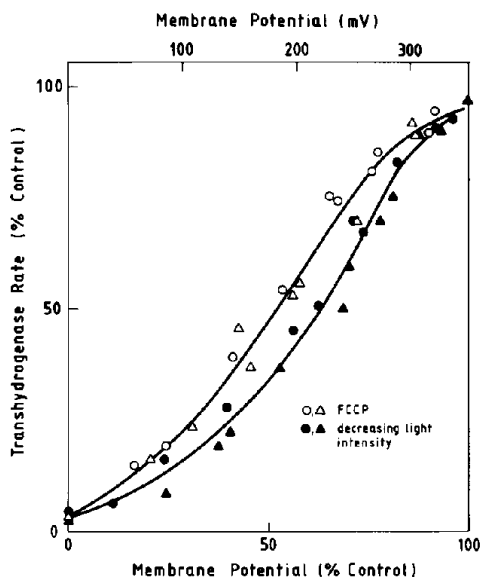


Fig.4. The dependence of transhydrogenase rate on  $\Delta\psi$  revealed either by titration with FCCP (at full light intensity) or by reducing the photosynthetic light intensity. The two sets of symbols ( $\circ, \bullet$  and  $\Delta, \blacktriangle$ ) describe the results of two sets of experiments carried out on different chromatophore preparations. Uninhibited rates and  $\Delta\psi$  values at maximum light intensity were normalised to 100%. Full light intensity ( $1.7 \times 10^6 \text{ erg} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$ , estimated with a silicon photodiode calibrated against a Hewlett-Packard thermopile) was close to saturation on the basis that an 86% reduction led to a decrease in transhydrogenase rate of only approx. 10%.

titrations with FCCP and with photosynthetic light intensity. Throughout the profile, for the same apparent value of  $\Delta\psi$ , the transhydrogenase rate was greater when FCCP was used to depress  $\Delta\psi$  than when reduced light intensity was employed.

#### 4. DISCUSSION

##### 4.1. The dependence of transhydrogenase rate on $\Delta\psi$

The equilibrium constant for the transhydrogenase reaction is close to unity. In the conditions of our experiments, the low rate of transhydrogenase activity in the 10 min preincubation period led to nucleotide concentrations of typically  $60 \mu\text{M}$  thio-NADP,  $126 \mu\text{M}$  NADH,

$7 \mu\text{M}$  thio-NADPH and  $7 \mu\text{M}$  NAD at the instant before illumination.  $\Delta G$  was therefore approx.  $-12 \text{ kJ} \cdot \text{mol}^{-1}$  and this increased to approx.  $-10 \text{ kJ} \cdot \text{mol}^{-1}$  during 10 s of illumination at the maximum transhydrogenase rate. Clearly the reaction is thermodynamically favourable even at  $\Delta\psi = 0$ . Evidently the threshold potential at about 50 mV is kinetic rather than thermodynamic. Whether this represents a  $\Delta\psi$ -dependent transition from an inactive to an active form of the transhydrogenase enzyme or whether it is a feature of the mechanism by which the protonmotive force drives catalysis remains to be seen.

##### 4.2. The unique relation between transhydrogenase rate and $\Delta\psi$

The dependence of the rate of transhydrogenase on  $\Delta\psi$  (when  $\Delta\text{pH} = 0$ ) was similar when either of two different electron transport inhibitors or an uncoupling agent were used to depress  $\Delta\psi$ . This result is consistent with the chemiosmotic hypothesis in its original form and does not provide any evidence for the existence of 'localised' interactions between the photosynthetic electron transport chain and the transhydrogenase enzyme (cf. [10]).

##### 4.3. Distortion of the profile between transhydrogenase rate and $\Delta\psi$ at reduced photosynthetic light intensity

The relationship between transhydrogenase rate and  $\Delta\psi$  was altered when the titration was performed by reducing the photosynthetic light intensity (fig.4). This might imply that the activity of the enzyme is modulated by chemical interactions with the photosynthetic apparatus. However, experiments at less-than-saturating light intensities should be interpreted with caution [15]. At bacteriochlorophyll concentrations routinely used in spectroscopic experiments (e.g.  $10 \mu\text{M}$ ), the actinic light intensity in the sample (e.g. in the region of 800 nm) decreases by about 99% per cm. Consequently chromatophores close to the actinic beam are exposed to a significantly higher light intensity than those further away. When the light intensity does not saturate photosynthesis then the light gradient inevitably leads to a population of chromatophores which are inhomogeneously energised. The probe beam, of finite width, at  $90^\circ$

to the actinic beam, records the sum of the absorbances by individual vesicles from such a population. Even when  $J_t$  and  $\Delta\psi$  are measured by probe beams at the same position in the sample, the true relationship can be appreciably distorted under these conditions of limiting light intensity. A precise analysis is complicated by the number of non-linear and poorly defined relationships involved in the causal chain of events: the dependence of light intensity on path length (which is a function of wavelength), the dependence of  $\Delta\psi$  generation on light intensity (which is also a function of wavelength) and the dependence of  $J_t$  on  $\Delta\psi$ . The effect of a non-saturating light gradient on a profile of  $J_t$  vs  $\Delta\psi$  which is characterised by saturation at large values of  $\Delta\psi$  will usually lead to a misleadingly steeper response. This might account for the result in fig.4. In general, investigations into the dependence of transhydrogenase rate or ATP synthesis rate or solute translocation rate on the protonmotive force, at light intensities which are less than saturating, could suffer from this complication. It only remains to point out that rather high light intensities are needed to saturate photosynthetic electron transport rates in photosynthetic bacteria like *Rb. capsulatus* (see fig.4). Since the experiments of figs 2 and 3 were carried out at saturating light intensities it is likely that these data describe the true dependence of  $J_t$  on  $\Delta\psi$ .

#### ACKNOWLEDGEMENT

J.F.M. was a recipient of a Science and Engineering Research Council research studentship.

#### REFERENCES

- [1] Sorgato, M.C., Lippe, G., Seren, S. and Ferguson, S.J. (1985) FEBS Lett. 181, 323–327.
- [2] Woelders, H., Putters, J. and Van Dam, K. (1986) FEBS Lett. 204, 17–21.
- [3] Portis, A.R. and McCartney, R.E. (1974) J. Biol. Chem. 249, 6250–6254.
- [4] Davenport, J.W. and McCartney, R.E. (1986) Biochim. Biophys. Acta 851, 136–145.
- [5] Zoratti, M., Pietrobon, D. and Azzone, G.F. (1982) Eur. J. Biochem. 126, 443–451.
- [6] Zoratti, M. and Petronilli, V. (1985) FEBS Lett. 193, 276–282.
- [7] Mandolino, G., De Santis, A. and Melandri, B.A. (1983) Biochim. Biophys. Acta 723, 428–439.
- [8] Baccarini-Melandri, A., Casadio, R. and Melandri, B.A. (1977) Eur. J. Biochem. 78, 389–402.
- [9] Casadio, R., Baccarini-Melandri, A. and Melandri, B.A. (1978) FEBS Lett. 87, 323–328.
- [10] Westerhoff, H.V., Melandri, B.A., Venturoli, G., Azzone, G.F. and Kell, D.B. (1984) Biochim. Biophys. Acta 768, 257–292.
- [11] Rydstrom, J. (1977) Biochim. Biophys. Acta 463, 15–184.
- [12] Keister, D.L. and Yike, N.J. (1966) Biochem. Biophys. Res. Commun. 24, 519–525.
- [13] Fisher, R.R. and Kaplan, N.O. (1973) Biochemistry 12, 1182–1188.
- [14] Cotton, N.P.J., Clark, A.J. and Jackson, J.B. (1981) Arch. Microbiol. 129, 94–99.
- [15] Clark, A.J., Cotton, N.P.J. and Jackson, J.B. (1985) Biochim. Biophys. Acta 723, 440–453.
- [16] 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/North-Holland, Amsterdam, New York.
- [17] Myatt, J.F., Cotton, N.P.J. and Jackson, J.B. (1987) Biochim. Biophys. Acta 890, 251–259.