TRANSHYDROGENASE-INDUCED RESPONSES OF CAROTENOIDS, BACTERIOCHLOROPHYLL AND PENETRATING ANIONS IN *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES

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1. Introduction

According to the chemiosmotic concept [1], the transmembrane gradient of electrochemical potential of the H^+ ions should be the driving force of oxidative and photosynthetic phosphorylation. Formation of an electric potential difference across coupling membranes has recently been demonstrated by several independent methods. In chloroplasts and bacterial chromatophores, appearance of a membrane potential can be detected by measuring shifts of absorption maxima of carotenoids and chlorophylls [2–5]. These spectral changes were demonstrated to be induced by light, hydrolysis of ATP or PP_i, oxidation of NADH by oxygen or oxidation of succinate by ferricyanide as well as by the diffusion potential of penetrating ions [3–5]. In *R. rubrum* chromatophores the differential spectrum of the membrane potential-induced spectral shift of carotenoids is characterized by maxima at 495, 535 and 570 nm, that of bacteriochlorophyll by the infrared shift with maxima at 795 and 910 nm and minima at 805 and 865 nm [4, 5].

The PCB^- probe is another method for measuring the membrane potential in *R. rubrum* chromatophores. It was shown [6] that this synthetic penetrating anion is taken up by energized chromatophores, the fact indicating formation of a membrane potential (positive inside) across the chromatophore membrane. Using this method the formation of membrane potential coupled with the forward transhydrogenase reaction ($NADPH + NAD^+ \rightarrow NADP^+ + NADH$) was discovered [6].

The present paper summarized the results of a further study of the latter phenomenon. To this end, three membrane potential probes were used: i) absorption changes of carotenoids, ii) those of bacteriochlorophyll and iii) transport of synthetic penetrating anions, PCB^-. It was shown that the forward transhydrogenase reaction induces characteristic changes in the parameters measured. The responses observed were found to be of the same direction as those induced by other energizing treatments, but of a smaller magnitude. The transhydrogenase-induced effects were specifically inhibited by the reaction products, $NADP^+$ and NADH. They were also sensitive to CCCP, a protonophorous uncoupler.

The similarity between responses of carotenoids, bacteriochlorophyll and PCB^- indicates that they are all caused by the electric field generated in the chromatophores oxidizing NADPH by NAD^+.

2. Methods

The procedure of preparing the *R. rubrum* chromatophores has been described elsewhere [6].

The carotenoid absorption changes at 570 nm and bacteriochlorophyll changes at 795 nm were measured with a two-wavelength spectrophotometer, Hitachi–356. The reference wavelengths were 590 nm and 800 nm for carotenoids and bacteriochlorophyll, respectively. The cuvette with the chromatophore suspension was thermostated at 20° in aerobic conditions.

Abbreviations:
CCCP, 2, 4, 6-trichlorocarbonyl cyanide phenylhydrazone; PCB^-, phenyldicarbaundecarborane; PP_i, inorganic pyrophosphate.

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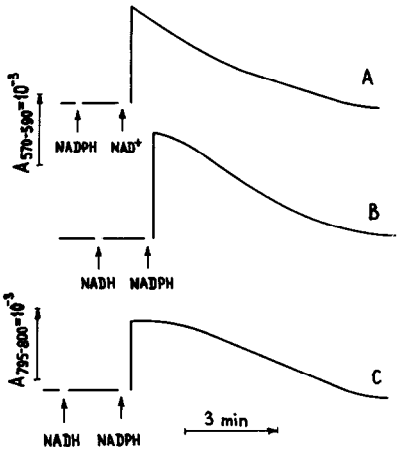

Fig. 1. The transhydrogenase-induced changes in absorption of carotenoids (A, B) and bacteriochlorophyll (C) in chromatophores of *R. rubrum*. Incubation mixture: rotenone 1×10^{-5} M (A, C) or 2×10^{-5} M (B); anitmycin A 1×10^{-6} M (A); chromatophores with contents of bacteriochlorophyll 30 μM (A), 61 μM (B) or 75 μM (C). In experiments B and C the medium was supplemented with 2 mM pyruvate and 0.06 mg/ml lactate dehydrogenase. Additions of nicotinamide nucleotides: 5×10^{-4} M (A, C) and 1×10^{-4} M (B).

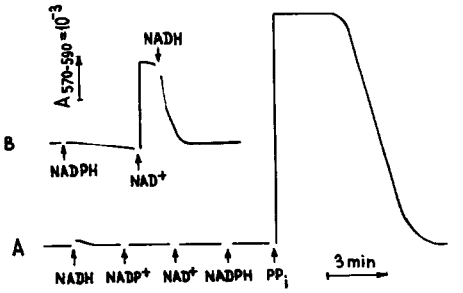

Fig. 2. The effect of NADH and $NADP^+$ on the carotenoid responses in chromatophores of *R. rubrum*. Incubation mixture: 2×10^{-6} M rotenone, 4×10^{-6} M antimycin A, chromatophores (61 μM bacteriochlorophyll). Additions: 5×10^{-4} M nicotinamide nucleotides, 7×10^{-5} M PP_i.

The PCB^- concentration changes were monitored with the phospholipid membrane technique [7].

The incubation mixture contained 0.25 M sucrose, 0.05 M Tris–HCl buffer (pH 7.8) and 5 mM $MgCl_2$. Concentrations of other components are indicated in the figure captions.

The concentration of bacteriochlorophyll was determined spectrophotometrically. The coefficient of molar extinction used was 140 $mM^{-1} \cdot cm^{-1}$ at 880 nm [8].

3. Results and discussion

It has been shown [4, 5] that the absorption of carotenoids at 570 nm and bacteriochlorophyll at 795 nm increases reversibly when *R. rubrum* chromatophores are energized. Fig. 1 demonstrates that effects of this type can be also observed after initiating the forward transhydrogenase reaction. Curve A shows a response of carotenoids. It is seen that an addition of NADPH is without effect; NAD^+, the second transhydrogenase substrate, induces an absorption increase. Apparently, this response is not due to the aerobic oxidation of NADH produced by the transhydrogenase reaction, because rotenone, an inhibitor of the chromatophore NADH dehydrogenase, was present in the incubation medium.

To exclude any possible effects of the NADH formed, the system was supplemented with lactate dehydrogenase and pyruvate. Fig. 1B and 1C show that NADH in the presence of rotenone, lactate dehydrogenase and pyruvate does not influence the absorption of carotenoids and bacteriochlorophyll. A subsequent addition of NADPH initiates reduction of pyruvate by the transhydrogenase–lactate dehydrogenase pair, inducing an effect similar to that in fig. 1A. In this case, the spontaneous decay is apparently due to the NADPH exhaustion.

As one can see in fig. 2A, pre-treatment of chromatophores with the products of the transhydrogenase reaction, NADH + $NADP^+$, completely prevents the increase in carotenoid absorption induced by NADPH + NAD^+, but not by pyrophosphate, another energizing agent used in this experiment. The absorption decreases to the initial level in 5 min, the time required for the whole portion of added pyrophosphate to be hydrolyzed.

One of the products of the transhydrogenase reaction, NADH, if added after the substrates, reverses the effect of the latter on the carotenoid absorption (fig. 2B). The product inhibition is a characteristic feature of any energy-dependent effects induced by the transhydrogenase reaction. In fact, transhydrogenase is a unique energy coupling site of the redox chain for

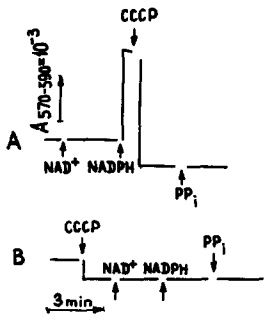

Fig. 3. The effect of the uncoupler CCCP on the carotenoid responses in the *R. rubrum* chromatophores. Incubation mixture: 1×10^{-6} M rotenone, 1.4×10^{-5} M antimycin A, chromatophores (61 μM bacteriochlorophyll). Additions: 5×10^{-4} M nicotinamide nucleotides, PP_i, 3×10^{-5} M (A) or 7×10^{-5} M (B), 1×10^{-6} M CCCP.

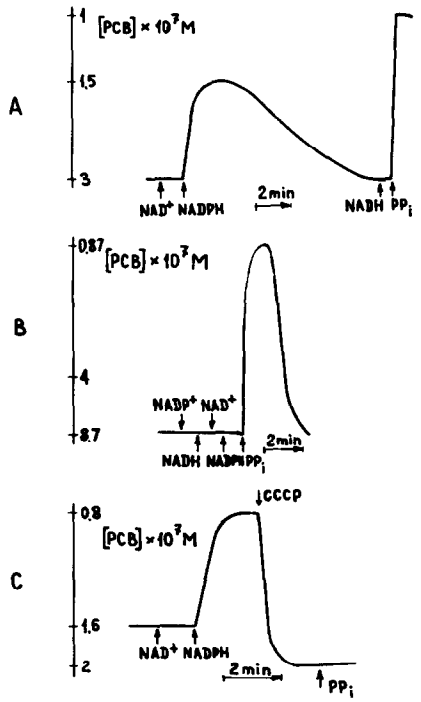

Fig. 4. Accumulation of the PCB^- anions by chromatophores of *R. rubrum*, induced by the forward transhydrogenase reaction and by PP_i hydrolysis. Incubation mixture: 2×10^{-5} M rotenone, 6×10^{-6} M antimycin A, chromatophores (61 μM bacteriochlorophyll in exps. A, C or 24 μM in exp. B). Additions: nicotinamide nucleotides 5×10^{-4} M (A, B) or 1×10^{-4} M (C); PP_i 1×10^{-4} M (A), 7×10^{-5} M (B) or 2×10^{-5} M (C); 3×10^{-7} M CCCP.

which the only energy source is an excess of the reaction substrate concentration over that of the products [6, 7, 9, 10].

It is known that protonophorous uncouplers of phosphorylation inhibit the energy-dependent spectral effects of carotenoids and bacteriochlorophyll in chromatophores [4, 5].

Fig. 3 indicates that an addition of CCCP abolishes both the transhydrogenase- and pyrophosphatase-linked carotenoid responses.

Fig. 4 demonstrates the transhydrogenase- and pyrophosphatase-induced responses of PCB^-, a penetrating anion.

Addition of NAD^+ and NADPH to a suspension of chromatophores results in a reversible decrease in the anion concentration in the medium (fig. 4A). NADH, if added on completion of the spontaneous decay of the transhydrogenase-linked response, is without effect. Addition of pyrophosphate causes a PCB^- uptake of a higher magnitude than the (NAD^+ + NADPH)-induced response. Addition of transhydrogenase products prior to the substrates prevents the effect of the latter but not of pyrophosphate (fig. 4B).

Fig. 4C shows that CCCP reverses the transhydrogenase-induced PCB^- response and prevents the pyrophosphate-induced one from taking place.

The similarity of the spectral effects of carotenoids and bacteriochlorophyll, on the one hand, and the effects of the penetrating anions, on the other, supports the idea that all these responses are induced by a membrane potential generated by the transhydrogenase-mediated transfer of reducing equivalents from NADPH to NAD^+.

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