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Received May 22nd, 1970
Revised manuscript received July 27th, 1970

BBA 43277

On the relationship of the energy-linked transhydrogenase to energy-linked NAD^+ reduction in *Rhodospirillum rubrum*

Chromatophores of *Rhodospirillum rubrum* catalyze a light-dependent reduction of NAD^+ using succinate[1] or other electron donors[2,3]. KEISTER AND YIKE[4] demonstrated that this reaction is sensitive to inhibitors and uncouplers of photophosphorylation. The latter authors concluded that NAD^+ reduction reflects reversed electron flow in the oxidative electron-transport chain supported by some high energy intermediate of photophosphorylation (Eqn. 1).

$$\text{Succinate} + NAD^+ + X \sim Y \leftrightharpoons \text{fumarate} + NADH + H^+ + X + Y \quad (1)$$

Another energy-linked reduction observed in *R. rubrum* chromatophores is the photoreduction of $NADP^+$ during energy-linked transhydrogenation. This reaction which required NADH as a specific electron donor, was found to be sensitive to uncouplers and inhibitors of phosphorylation[4,5] and has in a similar way been assumed to be driven by the energized intermediate of the conservation mechanism (Eqn. 2).

$$NADH + NADP^+ + X \sim Y \longrightarrow NAD^+ + NADPH + X + Y \quad (2)$$

Work from this laboratory[6,7] has shown that the system responsible for the catalysis of the energy-linked transhydrogenase of *R. rubrum* can be resolved into a soluble protein factor and an insoluble membrane component. The partially purified soluble protein is not itself a transhydrogenase, since it does not carry out the energy- or nonenergy-linked reaction in the absence of the membrane component[8]. It also does not influence the rate of light-induced ATP synthesis in transhydrogenase factor-resolved particles[6], indicating that it is not functional in the formation or stabilization

of X ~ Y. The question arises as to a possible relationship of the soluble transhydrogenase factor to the energy-dependent reduction of NAD^+. This communication reports on certain aspects of this question.

Chromatophores were prepared from photosynthetically grown *Rhodospirillum rubrum* (van Niel Strain-1) (ref. 9) by grinding with sand[10]. Bacteriochlorophyll was assayed according to the method of CLAYTON[11]. The energy-linked reduction of NAD^+ with succinate was assayed by the method of KEISTER AND YIKE[4], in a medium (3 ml) consisting of 50 mM Tris–HCl, pH 8; 150 mM sucrose; 1 mM $MgCl_2$; 0.10 mM NAD^+; 3.3 mM sodium succinate and chromatophores containing 20 μg of bacteriochlorophyll. The energy-linked transhydrogenase was assayed[10] using chromatophores containing 20 μg of bacteriochlorophyll.

Chromatophores prepared from 50 g (wet weight) of *R. rubrum* cells were suspended in 90 ml of 100 mM Tris–HCl, 10 % sucrose buffer, pH 8, at 0–4° and centrifuged at 150000 × *g* for 30 min. The washing procedure was repeated 2 additional times. The light-dependent transhydrogenase and NAD^+ photoreduction activities of the chromatophores were determined initially and following each washing. From Fig. 1, it is obvious that extensive washing leads to a complete loss of the membrane-catalyzed transhydrogenation. The loss of transhydrogenase activity represents a solubilization of a transhydrogenase factor from the chromatophore membrane[8]. On the other hand, it can be seen that the rate of the light-induced reduction of NAD^+ by succinate was not substantially influenced by the removal of the transhydrogenase activity.

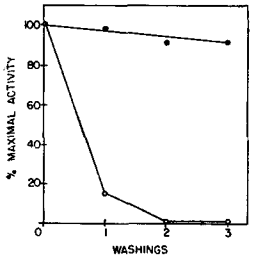

Fig. 1. The effect of washing on energy-linked transhydrogenase of *R. rubrum* chromatophores and the energy-linked reduction of NAD^+ with succinate. ○—○, energy-linked transhydrogenase; ●—●, energy-linked reduction of NAD^+ by succinate. Maximal activity was 41.5 μmoles $NADP^+$, reduced per mg bacteriochlorophyll per h for transhydrogenation, and 8.7 μmoles NAD^+, reduced per mg bacteriochlorophyll per h for the energy-linked reduction of NAD^+ by succinate. Light intensity was $9.5 \cdot 10^4$ ergs·cm^{-2}·sec^{-1} and the temperature 23°.

This distinction between the energy-linked transhydrogenase and the energy-linked reduction of NAD^+ in *R. rubrum* chromatophores is consistent with similar differences observed for the same energy-linked reactions of submitochondrial particles. KAWASAKI *et al.*[12] found that an antibody prepared against a digitonin-solubilized transhydrogenase enzyme from ox heart mitochondria inhibited the energy-linked transhydrogenase reaction in submitochondrial particles but not the energy-linked reduction of NAD^+ by succinate. LEE *et al.*[13] have eliminated the possibility that the transhydrogenase and the energy-dependent reduction of NAD^+ involve a common hydrogen transfer step requiring an energized pyridine nucleotide. The latter

investigators demonstrated that in submitochondrial particles the succinate-dependent reduction of NAD^+ involves the 4B hydrogen of NADH, whereas during transhydrogenation, $NADP^+$ is reduced by the 4A hydrogen of NADH.

It is concluded that the energy-linked reduction of NAD^+ with succinate and the energy-linked transhydrogenase reaction of *R. rubrum* chromatophores do not share the soluble transhydrogenase factor as a common component.

R.R.F. is a recipient of a predoctoral fellowship (5 FO 1 GM 3503) from the U.S. Public Health Service. R.J.G. is an established Investigator of the American Heart Association.

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Received June 23rd, 1970

Biochim. Biophys. Acta, 223 (1970) 204–206

BBA 43275

Cytochrome P-450 in adrenal mitochondria of male and female rats

Sex differences in adrenocortical function are well established. The adrenal is larger in the female, while adrenal venous corticosterone output is approximately twice as high in the female rat as in the male[1]. Liver homogenates from female rats reduce steroid ring A 3–10 times faster than those from males[2]. *In vitro* hydroxylation of 11-deoxycorticosterone to 18-hydroxy-11-deoxycorticosterone and corticosterone is stimulated by acute administration of estradiol *in vivo*[3], and this effect is apparently antagonized by testosterone *in vivo*[4].

Cytochrome P-450 acts as the terminal oxidase in many NADPH-dependent hydroxylation reactions involving steroids and drugs[5–7] including the 18- and 11β-hydroxylations of 11-deoxycorticosterone[8], which take place in adrenal cortex mitochondria[9]. The present study was undertaken to see what relationship exists between