A SOLUBLE TRANSHYDROGENASE ENZYME FROM *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES

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1. Introduction

Chromatophores isolated from the photosynthetic bacterium *R. rubrum*, catalyze the energy-linked reduction of $NADP^+$ by NADH [1] (eq. 1).

$$NADH + NADP^+ + X \sim Y \longrightarrow NAD^+ + NADPH + X + Y \quad (1)$$

In this representation, $X \sim Y$ is an energized intermediate of the energy conservation system.

In addition to the essentially irreversible energy-linked transhydrogenation, chromatophore membranes catalyze the nonenergy-linked reduction of NAD^+ (or, operationally, 3-AcPyAD$^+$) by NADPH (eq. 2).

$$NADPH + NAD^+ \text{ (or 3-AcPyAD}^+\text{)} \rightleftharpoons NADP^+ + NADH \text{ (or 3-AcPyAD·H)} \quad (2)$$

Previous work from this laboratory [2–4] has demonstrated that extensive washing of chromatophore membranes of *R. rubrum* results in the loss of both energy-linked and nonenergy-linked transhydrogenase activities. A protein factor, which is able to reconstitute transhydrogenation in these resolved particles, has been isolated and partially purified from cell-free extracts.

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Abbreviations: 3-AcPyAD$^+$, 3-Acetylpyridine adenine dinucleotide; 3-AcPyAD·H, 3-Acetylpyridine adenine dinucleotide reduced form; BChl, bacteriochlorophyll.

The energy-dependent reduction of $NADP^+$ by NADH (eq. 1) and the nonenergy-linked reduction of 3-AcPyAD$^+$ by NADPH (eq. 2) were stimulated in resolved particles to an identical percent of their respective maximum rates at each concentration of added purified factor. This finding indicated that transhydrogenase factor is required for both energy-linked and nonenergy-linked transhydrogenation and that the binding site for transhydrogenase factor on the chromatophore membrane is identical for both the energy-linked and nonenergy-linked reaction.

2. Materials and methods

Photosynthesizing cultures of *R. rubrum* were harvested and chromatophores prepared by sonication as previously described [5]. A crude preparation of transhydrogenase factor was isolated following $(NH_4)_2SO_4$ precipitation of the supernatant remaining after the initial 150 000 *g* sedimentation of the chromatophores. A transhydrogenase factor preparation of greater purity was obtained as previously reported [6] by washing the chromatophores, three times with 0.1 M Tris–HCl, pH 8, 1% sucrose and precipitating the protein present in the washings with $(NH_4)_2SO_4$. Table 1 shows that the solubilized transhydrogenase factor itself was able to catalyze a second type of nonenergy-linked reduction of 3-AcPyAD$^+$ by NADH while the reduction of 3-AcPyAD$^+$ by NADPH has an absolute requirement for both the resolved chromatophore membrane as well as transhydrogenase factor.

Further attempts to characterize the membrane binding site of the transhydrogenase factor revealed

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Table 1
The catalysis of nonenergy-dependent transhydrogenation by *R. rubrum* transhydrogenase factor

	μmoles 3-AcPyAD$^+$ reduced/hr	
Additions	NADPH	NADH
3-AcPyAD$^+$	—	—
$^+$ Transhydrogenase Factor	—	.35
$^+$ Resolved Chromatophores	.78	.42

The reaction medium (3 ml) contained 43 mM Tris–HCl, pH 8, 123 mM sucrose, 0.7 mM $MgCl_2$, 0.0085 mM rotenone. When added, NADPH and NADH were 0.067 mM, 3-AcPyAD$^+$ was 0.134 mM, transhydrogenase factor (20 μl) contained 0.13 mg of protein, and resolved chromatophores 10 μg BChl.

that both the energy-linked reduction of NADP$^+$ by NADH and the nonenergy-linked reduction of 3-AcPyAD$^+$ by NADPH were inhibited following treatment of the resolved chromatophores with phospholipase A, the antibiotic Dio-9, as well as with oleyl- and palmitoyl-CoA (Myers and Guillory, submitted for publication). This was in contrast to results obtained when NADH was used as a reductant for 3-AcPyAD$^+$. This latter transhydrogenase activity was insensitive to treatment with phospholipase A, Dio-9,

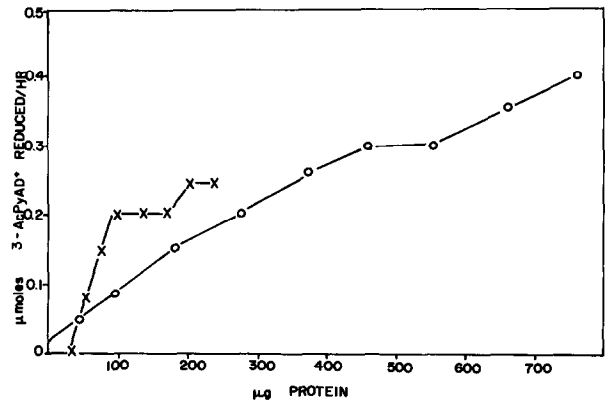

Fig. 1. The rate of 3-AcPyAD$^+$ reduction by NADH in the presence of a crude(o) or wash(x) preparation of transhydrogenase factor. Assay conditions as for table 1, but without chromatophore; the transhydrogenase preparations were dialyzed against 0.01 M Tris–HCl, pH 8, 1% sucrose, and 100 μM NADP$^+$ prior to use; NADH was present at 0.067 mM.

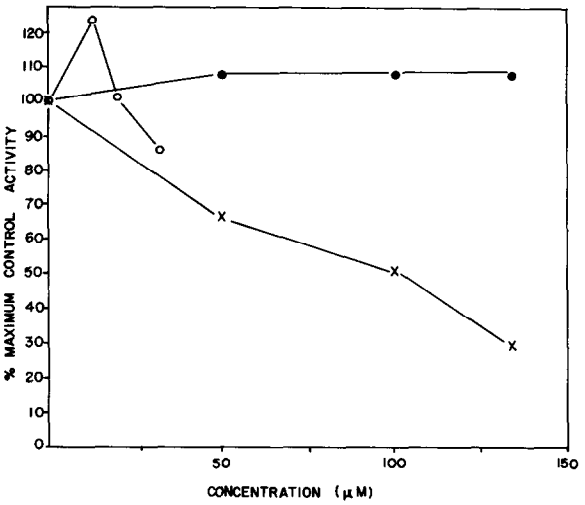

Fig. 2. The influence of NADP$^+$(·), NAD$^+$(x), and palmitoyl-CoA(o) on the nonenergy-linked reduction of 3-AcPyAD$^+$ by NADH. Assay conditions as for table 1 with 20 μl (0.13 mg) of transhydrogenase factor (wash preparation) present.

as well as oleyl- and palmitoyl-CoA. Results shown in fig.1 indicate that the nonenergy-linked reduction of 3-AcPyAD$^+$ by NADH is proportional to the concentration of transhydrogenase factor and that factor preparations obtained from chromatophore washes have a higher specific activity than the crude preparation.

On the basis of the stabilizing influence of NADH on the transhydrogenase factor [4] we had postulated that the solubilized transhydrogenase factor contains the NAD(H) binding site while the resolved chromatophore membrane is required for NADP(H) binding. The data presented in fig. 2 shows that the addition of NAD$^+$ to the assay system inhibits the reduction of 3-AcPyAD$^+$ by NADH, presumably by NAD$^+$ competition with 3-AcPyAD$^+$ for the hydride ion. NADP$^+$, on the other hand, shows no inhibitory effect on the reaction. These experiments carried out in the absence of the chromatophore membrane tend to support our hypothesis concerning the locus of NAD(H) interaction during the catalysis of transhydrogenation.

3. Discussion

It is our working hypothesis that our isolated transhydrogenase factor contains the catalytic site for

NAD(H) while the chromatophore membrane retains the NADP(H) binding site for transhydrogenation. The new finding that the partially purified transhydrogenase factor can catalyze the nonenergy-linked reduction of 3-AcPyAD$^+$ by NADH provides a useful took for the further purification and characterization of the factor. It is significant that the factor is not able to catalyze independent of the chromatophore membrane, any of the other energy- or nonenergy-linked transhydrogenations. Present attempts in this laboratory at the isolation and characterization of the membrane-bound NADP(H) site may well allow a determination of the mechanism by which the protein transhydrogenase factor results in activation of energy-dependent transhydrogenation.

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