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THE EFFECT OF AGING RESOLVED CHROMATOPHORES OF *RHODOSPIRILLUM RUBRUM* ON THE CAPACITY TO RECONSTITUTE THE ENERGY-LINKED TRANSHYDROGENATION

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SUMMARY

Chromatophores which are resolved with respect to energy-linked transhydrogenation can be reconstituted with a soluble transhydrogenase factor. A decrease in the light-stimulated activity is found with transhydrogenase factor of increasing purity while the ATP- and pyrophosphate-driven reactions are maintained at their maximum rate. The inability to fully reconstitute the light-driven transhydrogenase activity has been found to be due to a reversible alteration within the resolved chromatophore membrane. Evidence is presented which leads to the postulate that the aging process damages those membrane sites which involve non-cyclic electron transport, are not associated with phosphorylation, and are involved in driving 50 % of the light-dependent transhydrogenation.

An energy-dependent reduction of $NADP^+$ by NADH is catalyzed by chromatophores of *Rhodospirillum rubrum*. Work from this laboratory[1-3] has shown that the system responsible for this transhydrogenation can be resolved into a soluble protein factor and an insoluble membrane component. The soluble protein factor does not catalyze transhydrogenation in the absence of the membrane component, but in its presence restores transhydrogenation to the level of activity observed in unresolved chromatophores. Transhydrogenation in both unresolved[4] and the reconstituted systems[2] can be driven by light, ATP, and pyrophosphate. The maximum rate of the light-driven reaction is of the order of twice that found for the maximal ATP- or pyrophosphate-driven reaction[2,4].

While the rate of the light-driven transhydrogenation in resolved chromatophores can be fully restored by the use of a crude transhydrogenase factor[1], it was observed that further purification of the factor could lead to a decrease in the maximum degree of light-stimulated activity[5]. On the other hand the ATP- and pyrophosphate-driven reactions were fully reconstituted to their maximal activity with either preparation.

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These observations led us to postulate as a working hypothesis the possible existence of a second factor involving specifically the light-driven transhydrogenase reaction. The experiments reported in this communication indicate that the inability to fully reconstitute the light-driven transhydrogenase reaction is not due to the loss of a soluble transhydrogenase factor component, but rather to an altered condition of the resolved chromatophore. This deleterious modification of the membrane upon resolution can be corrected by the addition of dithiothreitol.

Chromatophores were prepared from photosynthetically grown *R. rubrum* (Van Neil strain-1)[6] by sonication for 1 min at 4° using a Branson model J32 sonic oscillator[2]. The chromatophore fraction consisted of those particles sedimenting between $10000 \times g$ and $150000 \times g$ for 30 min. The protein obtained from the $150000 \times g$ supernatant by precipitation with $(NH_4)_2SO_4$ between 40 and 80 % saturation at 0° is designated the crude supernatant fraction. A protein fraction of higher specific activity was obtained by washing chromatophores with 0.1 M Tris–HCl, pH 8.0, 10 % sucrose and collecting the protein precipitating between 40 and 90 % $(NH_4)_2SO_4$ saturation; this fraction is referred to as the wash fraction. The chromatophores were washed repeatedly until completely free of energy-linked transhydrogenase activity[1]. Aging of the resolved chromatophores was performed by placing the particles in a test tube and immersing this in a water bath at 37° for various periods of time. Bacteriochlorophyll concentration was assayed according to the method of CLAYTON[7] and transhydrogenase activity was determined by continuously monitoring the absorbance at 340 nm in a Gilford Spectrophotometer using the assay medium of KEISTER AND YIKE[4]. Direct illumination of the chromatophores was achieved by means of a fiber glass optical rod (American Optical Company) inserted through a hole in the top of the sample compartment and positioned directly above one of the cuvettes. A filter was used to restrict light of less than 620 nm from the sample. A second filter positioned between the sample and phototube cut off light above 400 nm.

Table I shows that when the crude supernatant fraction is used to reconstitute transhydrogenation in aged chromatophores, there is only a minimal effect of aging. If, however, the partially purified wash fraction is used for the reconstitution of these resolved chromatophores, the ability to reconstitute maximal light-driven transhydrogenase is decreased some 50 % following a 30-min aging period. Aging of chromatophores for periods in excess of 30 min did not further decrease the ability of the partially purified wash fraction to reconstitute transhydrogenation. The ATP-driven transhydrogenation was not affected by the aging of the resolved chromatophores irrespective of the transhydrogenase factor used for reconstitution. At saturating levels of either transhydrogenase factor preparation, both the light- and ATP-stimulated rates are identical in chromatophores aged for at least 30 min, and remains fairly constant upon further aging of the chromatophore membrane. The addition of 1 mM dithiothreitol together with the wash fraction to the aged chromatophores completely restores the light-driven reaction.

Resolved and subsequent aged chromatophores were tested for oligomycin-sensitive ATPase, and the photophosphorylation of ADP. No major loss was seen in these activities when compared to resolved particles which had not been aged[8].

The complete reconstitution of transhydrogenation using the wash fraction in the presence of dithiothreitol indicates that this factor preparation is still able to completely reconstitute light-driven transhydrogenation and that there is no reason

TABLE I

RECONSTITUTION OF ENERGY-LINKED TRANSHYDROGENATION BEFORE AND AFTER AGING OF THE RESOLVED CHROMATOPHORES

The reaction medium contained 44 mM Tris–HCl, pH 8.0, 12.5 mM sucrose, 180 mM ethanol, 0.67 mM NADH, 1.67 mM $NADP^+$, 175 units of yeast alcohol dehydrogenase (Sigma), 10 mM $MgSO_4$ and chromatophores containing 10 μg bacteriochlorophyll. In the ATP-driven reaction 2.5 mM ATP replaced light. Saturating amounts of the factor preparation were used in these assays, the specific activities of the crude supernatant factor and the wash preparations were 1.5 and 42.8 μmoles NADPH formed per mg protein per h, respectively. Illumination was at $7.5 \cdot 10^4$ ergs$\cdot$cm$^{-2}\cdot$sec^{-1}.

Additions	*Transhydrogenation (μmoles NADPH formed/mg bacteriochlorophyll per h)*	
	Light	*ATP*
Chromatophore, control:		
—	5	3
Crude supernatant	105	55
Wash supernatant	108	60
1 mM dithiothreitol	5	5
Wash supernatant + 1 mM dithiothreitol	105	55
Chromatophore, aged 1 h at 37°:		
Crude supernatant	85	40
Wash supernatant	45	45
Wash supernatant + 1 mM dithiothreitol	90	45
Dithiothreitol	3	4

to assume the existence of two different transhydrogenation factors. The decrease in the light-stimulated activity with increasing purity of the transhydrogenase factor represents an effect upon the chromatophore membrane as a result of resolution. If resolved chromatophores are kept at 4° for more than 48 h, the capacity to reconstitute the light-driven transhydrogenase reaction is reduced 50 % using the wash factor preparation. Fig. 1 illustrates the dithiothreitol dependency for the reconstitution of

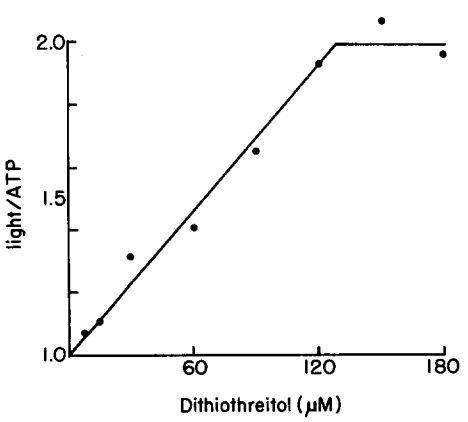

Fig. 1. Reconstitution of energy-linked transhydrogenation by transhydrogenase factor and dithiothreitol. The transhydrogenation was assayed as described in Table I. The resolved chromatophore particles used were aged for 120 min at 37°.

transhydrogenation using aged chromatophores and the wash transhydrogenase fraction. Complete restoration of the maximum transhydrogenation rate is found at a dithiothreitol concentration in excess of 120 μM.

Evidence exists for the presence of two distinct light receptor units in photosynthetic bacteria[8–10]. In *R. rubrum* chromatophores one of the units is postulated as consisting of a light-driven electron transport system which is cyclic and phosphorylating, while the second light-driven electron system operates at a lower potential and is non-cyclic and non-phosphorylative[8]. Presumably the transhydrogenase reaction can be driven by the energy derived from both of these pathways.

Unresolved chromatophores are not affected by the aging procedure described in this report (A. W. T. KONINGS AND R. J. GUILLORY, unpublished results), possibly because of the protection afforded by a factor which is removed during resolution, rendering the chromatophore vulnerable to oxidation. It is unlikely that this protective agent is the transhydrogenase factor itself since the purified transhydrogenase factor does not protect against aging (A. W. T. KONINGS AND R. J. GUILLORY, unpublished results). Since no major changes occur in the oligomycin-sensitive ATPase or in the rate of photophosphorylation of ADP under the conditions described for the aging of resolved particles, we consider that the aging process may damage selectively those sites on the chromatophore membrane which involve non-cyclic electron transport, are not associated with phosphorylation and drive 50 % of the total light-dependent transhydrogenation.

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