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SPECIFICITY OF THE TRANSHYDROGENASE FACTOR FOR CHROMATOPHORES OF *RHODOPSEUDOMONAS SPHEROIDES* AND *RHODOSPIRILLUM RUBRUM*

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SUMMARY

Extensive washing of chromatophores of *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* with dilute buffer results in a complete loss of the energy-linked transhydrogenase activities of *Rsp. rubrum* but only a partial loss of the light-driven reaction in chromatophores of *Rps. spheroides*. It was not possible to reactivate the *Rps. spheroides* transhydrogenation with the *Rsp. rubrum* transhydrogenase factor nor with a protein fraction of *Rps. spheroides* isolated by procedures identical to that used for the isolation of the *Rsp. rubrum* transhydrogenase factor. The *Rsp. rubrum* factor is highly specific and cannot be replaced by a number of sulfhydryl compounds tested for reconstitution of *Rsp. rubrum* transhydrogenation. A published procedure for the isolation of a "transhydrogenase factor" from *Rps. spheroides* chromatophores yields a preparation having energy-dependent transhydrogenation when supplemented with dithiothreitol in the absence of added chromatophores.

INTRODUCTION

Energy-dependent transhydrogenase reactions have been described for the photosynthetic bacteria, *Rhodospirillum rubrum*[1] and *Rhodopseudomonas spheroides*[2]. The energy-dependent characteristics of the bacterial reactions are similar to those of the ATP-dependent transhydrogenation described for mitochondria[3].

Extensive washing of *Rsp. rubrum* chromatophores with dilute buffer solution results in a complete loss of their ability to catalyze transhydrogenation[4]. Reconstitution of this activity with the resolved *Rsp. rubrum* membrane is dependent upon the addition of a soluble protein isolated from the chromatophore washings[5,6]. This soluble transhydrogenase factor does not catalyze transhydrogenation in the absence of the resolved membrane[6]. The factor functions in both the energy- and the non-energy-linked transhydrogenation[5].

An apparent resolution has been reported for the transhydrogenase reaction

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of *Rps. spheroides*[7]. Since reconstitution of the *Rps. spheroides* transhydrogenase could be accomplished by the addition of sulfhydryl reagents such as dithiothreitol, dithioerythritol and reduced thiocitic acid, it was felt that the specificity of the transhydrogenase factor isolated from *Rsp. rubrum* was open to question.

In this paper we present evidence indicating that (a) the transhydrogenase factor as isolated from *Rsp. rubrum* chromatophores is obligatory for the transhydrogenation reaction in depleted *Rsp. rubrum* chromatophore membranes and cannot be substituted for by dithiols, (b) the data on the transhydrogenase reaction in chromatophores of *Rps. spheroides* can be interpreted as an inactivation of the chromatophore membrane rather than a true resolution, (c) the method used for the resolution of transhydrogenation in *Rsp. rubrum* can not be directly applied for the resolution of the transhydrogenation reaction in *Rps. spheroides*.

MATERIALS AND METHODS

Rhodospirillum rubrum (S-1) was grown photosynthetically in the medium of Ormerod *et al.*[8] supplemented with 0.1 % yeast extract. *Rhodopseudomonas spheroides* was grown in a modified Hunter medium[9]. The cells were cultured in completely filled 9-l bottles irradiated by four 100-W tungsten bulbs placed 2 ft from the bottles. The cells were harvested after 52 h by centrifugation at 600 × *g*, washed once with 0.01 M Tris–HCl (pH 8), 1 % sucrose and stored at −20 °C.

Chromatophores were prepared by a 30-s sonication at 4 °C using a Bronson Model J 32 sonic oscillator[6] and collecting those particles sedimenting between 18000 × *g* and 150000 × *g* after 60 min. The *Rsp. rubrum* transhydrogenase factor as well as the "factor" (I) from *Rps. spheroides* was a dialyzed preparation obtained by $(NH_4)_2SO_4$ (40–80 % saturation at 0–4 °C) precipitation of the protein in the 150000 × *g* supernatant. Washing of the chromatophores was carried out as previously described[4–6]. Bacteriochlorophyll was measured as described by Clayton[10]. Protein was determined by the method of Lowry *et al.*[11] using serum albumin as a standard. Reconstituted energy-linked transhydrogenation, pyrophosphatase and ATPase activities were assayed as previously described[12]. For the pyrophosphatase measurement 20 μg of bacteriochlorophyll for *Rsp. rubrum* or 4 μg bacteriochlorophyll for *Rps. spheroides* were used. Phosphate was determined according to the method of Lohman and Jendrassik[13, 14].

RESULTS AND DISCUSSION

Table I shows that extensive washing of chromatophores of *Rsp. rubrum* completely resolved the bacterial membrane with respect to energy-linked transhydrogenation[4]. It is obvious that washed chromatophores of *Rps. spheroides* retain a major portion (65 %) of the light-driven transhydrogenase reaction. While the *Rsp. rubrum* transhydrogenase activity could be fully reconstituted with the transhydrogenase factor, attempts to reconstitute the lost *Rps. spheroides* transhydrogenase activity using a preparation from the cell-free extract of this bacterium (obtained in the same way as for *Rsp. rubrum*) were not successful. It was also impossible to reconstitute transhydrogenation in resolved *Rsp. rubrum* chromatophores with the *Rps. spheroides* protein (not shown).

TABLE I

RESOLUTION AND RECONSTITUTION OF THE TRANSHYDROGENATION REACTION IN CHROMATOPHORES OF *Rsp. rubrum* AND *Rps. spheroides*

The transhydrogenase factor from *Rsp. rubrum* and the "factor preparation" (I) from *Rps. spheroides* were prepared as described in the text. The chromatophores were washed 4 times with 0.1 M Tris–HCl (pH 8), 10% sucrose and transhydrogenation was measured in a total volume of 3 ml as previously described[5,6] with chromatophores containing 10 μg bacteriochlorophyll. Saturating quantities of transhydrogenase factor were used with chromatophores of *Rsp. rubrum* (specific activity of 1.2 μmoles NADPH formed/mg protein per h), while 1.5 mg of protein was used in the experiments with *Rps. spheroides*. Dithiothreitol, when added, was used at a concentration of 1 mM. Transhydrogenation is recorded as μmoles of NADPH formed/mg bacteriochlorophyll per h.

Additions to the assay	*Transhydrogenase activity*			
Energy source:	*None*	*Light*	*ATP*	PP_i
Rsp. rubrum:				
Chromatophores	8	112	52	54
Washed chromatophores	7	5	7	7
Factor preparation	0	0	0	0
Transhydrogenase factor + dithiothreitol	0	0	0	0
Washed chromatophores + dithiothreitol	9	10	8	8
Washed chromatophores + transhydrogenase factor	9	108	51	50
Washed chromatophores + dithiothreitol + transhydrogenase factor	7	110	49	47
Rps. spheroides:				
Chromatophores	34	153	89	26
Washed chromatophores	21	98	23	15
Factor preparation (I)	0	0	0	0
Factor preparation (I) + dithiothreitol	0	0	0	0
Washed chromatophores + dithiothreitol	23	99	24	17
Washed chromatophores + factor preparation (I)	24	99	23	16
Washed chromatophores + dithiothreitol + factor preparation (I)	24	98	24	16

These experiments indicate that extensive washing of *Rps. spheroides* chromatophores does not result in the resolution of a transhydrogenase factor from the membrane resembling that factor isolated from *Rsp. rubrum* chromatophores.

If there is a transhydrogenase factor in *Rps. spheroides* similar to that found for *Rsp. rubrum* it must be tightly bound to the bacterial membrane. A similar situation appears to exist with regard to the mitochondrial transhydrogenase activity. Table I also shows that the transhydrogenase factor is very specific for the reconstitution of activity in chromatophores of *Rsp. rubrum* and cannot be replaced by dithiothreitol.

Assay of transhydrogenation following each washing revealed that almost the complete ATP-driven transhydrogenase reaction in *Rps. spheroides* is lost following one washing (not shown), under the same conditions where *Rsp. rubrum* chromatophores lose less than 50 % of the ATP-driven reaction[6].

From Table II it can be seen that neither the ATPase nor pyrophosphatase activities of the chromatophore membrane are affected by the washing procedure. These data suggest that washing of *Rps. spheroides* chromatophores with dilute buffer damages the ATP-driven and a portion of the light-driven mechanism of transhydrogenation rather than resolving from the membrane a factor required for the transhydrogenation. Even much more extensive treatment of the membrane failed to

TABLE II

EFFECT OF WASHING ON THE ATPase AND PYROPHOSPHATASE ACTIVITIES OF CHROMATOPHORES OF *Rsp. rubrum* AND *Rps. spheroides*

Chromatophores were prepared and washed as described in the text. Pyrophosphatase and ATPase activities were assayed as previously described[12] but in the absence of carbonyl cyanide *m*-chlorophenylhydrazone. Activity is expressed as μmoles of phosphate liberated/mg bacteriochlorophyll per h.

Addition	*Pyrophosphatase*	*ATPase*
Rsp. rubrum:		
Chromatophores	188	76
Washed chromatophores	204	82
Rps. spheroides:		
Chromatophores	2070	104
Washed chromatophores	1987	110

achieve a complete loss of membrane-bound transhydrogenase activity. A 1-min sonication of washed *Rps. spheroides* chromatophores in 0.01 M Tris–HCl (pH 8), 1 mM EDTA at 4 °C followed by 30 min incubation at 30 °C resulted in a complete loss of membrane-bound ATPase activity while 30 % of the light-driven transhydrogenase activity was still present.

Interestingly the pyrophosphatase activity in *Rps. spheroides* chromatophores is 10-fold higher than that measured for *Rsp. rubrum* chromatophores (Table II). The relatively high level of pyrophosphatase activity may explain the inability to catalyze a pyrophosphate-dependent transhydrogenation in *Rps. spheroides*.

Attempts were made to isolate a transhydrogenase factor preparation from *Rps. spheroides* by a procedure outlined in the literature[7]. As can be seen from Table III such a preparation has a light-driven transhydrogenase activity of its own in the presence of dithiothreitol. This activity was clearly inhibited by carbonyl cyanide *m*-chlorophenylhydrazone. It is our interpretation that this preparation contains

TABLE III

EFFECT OF DITHIOTHREITOL ON THE TRANSHYDROGENASE FACTOR PREPARATION OF *Rps. spheroides*

The *Rps. spheroides* "factor" was prepared from a 37000 × *g* supernatant of a cell suspension broken by a French pressure cell. The proteins precipitated by $(NH_4)_2SO_4$ between 45 and 75% saturation at 4 °C were dialized and used directly as described[7]. When added, dithiothreitol was at 1 mM and carbonyl cyanide *m*-chlorophenylhydrazone was at 1 μM. Transhydrogenation is recorded as μmoles of NADPH formed/mg bacteriochlorophyll per h.

Additions to the assay mixture	*Transhydrogenase activity*			
Energy source:	*Endogenous*	*Light*	*ATP*	PP_i
Factor preparation (1 mg)	3	5	2	2
Factor preparation + dithiothreitol	5	28	3	3
Factor preparation + dithiothreitol + carbonyl cyanide *m*-chlorophenylhydrazone	2	12	3	3

small membrane fragments and that these particles are reversibly inactivated during the precipitation with $(NH_4)_2SO_4$. Reactivation of the light-driven transhydrogenation in aged *Rsp. rubrum* chromatophores by dithiothreitol as shown here has been reported recently[15]. The experiments outlined with *Rps. spheroides* lend further support to the possible existence of two separate pathways for the energy-linked transhydrogenase, each with different susceptibilities to chemical or physical treatment.

We have found it possible to inactivate the reconstituting capacity of the *Rsp. rubrum* transhydrogenase factor with *p*-chloromercuribenzoate[16]. Reversability of the *p*-chloromercuribenzoate inhibition can be achieved by dithiothreitol. It is clear that the transhydrogenase factor is a distinct sulfhydryl protein up to now specific for the reconstitution of transhydrogenation in chromatophores of *Rsp. rubrum*.

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