JOURNAL OF BACTERIOLOGY, May 1971, p. 700-701

Vol. 106, No. 2
Printed in U.S.A.

Survey of the Photosynthetic Bacteria for Rhodanese (Thiosulfate:Cyanide Sulfur Transferase) Activity

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Received for publication 21 January 1971

Rhodanese activity was demonstrated in extracts from all three taxonomic families of photosynthetic bacteria, and this activity appeared to be uncorrelated with thiosulfate metabolism.

Rhodanese (thiosulfate:cyanide sulfur transferase, EC 2.8.1.1) catalyzes the cleavage of thiosulfate (equation 1).

$$CN^- + S_2O_3^{2-} \rightarrow CNS^- + SO_3^{2-} \quad (1)$$

This enzyme is widely distributed, having been detected in liver tissue, plant roots, and bacteria (4). In photosynthetic bacteria, rhodanese activity was first demonstrated in *Chromatium* (5) and was later found in *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum* (6). Rhodanese may play a role in thiosulfate oxidation in *Chromatium* by cleaving the sulfur-sulfur bond of thiosulfate to yield sulfur and sulfite (6).

The rhodanese activity of the three taxonomic families of photosynthetic bacteria was surveyed to determine if its occurrence is consistent with its proposed role in thiosulfate oxidation.

The organisms used in this study were cultured in the appropriate media as described by Bose (1) with only minor modifications. Extracts were prepared from a cell paste suspended in 25 mM tris(hydroxymethyl)aminomethane - hydrochloride buffer [*p*H 7.8 (1:3 w/v)], disrupted by sonic oscillation, and centrifuged as indicated in Table 1. Rhodanese activity was determined by the method of Smith and Lascelles (6), which is based on the *N*-methylphenazonium-methosulfate (PMS)-mediated reduction of 2,6-dichlorophenol indophenol (DPIP) by sulfite (equation 2) which results from the enzymatic cleavage of thiosulfate (equation 1).

$$SO_3^{2-} + DPIP \xrightarrow{PMS} SO_4^{2-} + DPIPH_2 \quad (2)$$

Protein concentration was measured by the Folin phenol method (2).

This reduction of DPIP by extracts of *Rhodopseudomonas palustris* was linear with time for 1 min and was proportional to added protein up to about 0.4 mg of protein per 3 ml. Except for a variation in rates, extracts of the other photosynthetic bacteria used gave similar results.

Table 1 compares the rhodanese activity in the crude extract with that of the supernatant and chromatophore fractions from a number of pho-

TABLE 1. *Rhodanese activity in photosynthetic bacteria*

Taxonomic family	Source of extract	Specific activity[a]		
		Crude extract (supernatant 30,000 × *g*)	Soluble extract (supernatant 105,000 × *g*)	Chromatophores (pellet 105,000 × *g*)
Athiorhodaceae	*Rhodospirillum rubrum*	88	171	9
	Rhodopseudomonas capsulata	68	120	0
	R. palustris	185	350	12
Thiorhodaceae	*Chromatium* sp.	107	170	9
	Ectothiorhodospira mobilis	116	117	8
Chlorobacteriaceae	*Chlorobium thiosulfatophilum*	36	55	5
	Chloropseudomonas ethylica	209	121	187[b]

[a] Nanomoles of DPIP reduced per minute per milligram of protein. The complete mixture contained: Tris buffer (*p*H 8.7), 300 μmoles; sodium thiosulfate, 150 μmoles; half neutralized sodium cyanide (NaCN/HCl, 2.5/1, mole/mole), 80 μmoles; DPIP, 0.5 μmole; and PMS, 0.25 mg. The protein concentration was 0.1 to 0.4 mg, and water was added to a final volume of 3.0 ml.

[b] Chromatophores were washed three times with 0.025 M Tris, *p*H 7.8.

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tosynthetic bacteria. All photosynthetic bacteria tested had rhodanese activity, suggesting that this enzyme is common to all species of this group. However, rhodanese does not appear to be associated with all photosynthetic tissue, as it was not observed in extracts of the blue-green alga *Anabaena cylindrica* or in spinach chloroplasts (specific activities <1). With one exception, rhodanese was found only in the supernatant fraction after centrifugation at $105{,}000 \times g$ for 1 hr (Table 1). In *Chloropseudomonas ethylica* extracts, activity was observed in both the soluble and the chromatophore fractions. Washing the chromatophores three times with 0.025 M Tris buffer did not diminish this activity. The reason for a chromatophore-bound rhodanese in this particular organism is not clear.

The occurrence of rhodanese activity in photosynthetic bacteria is apparently not correlated either with the ability to metabolize thiosulfate or with the route of thiosulfate metabolism. Rhodanese may be involved in thiosulfate metabolism in *Chromatium* (6), may not be in *R. palustris* (3, 7), and is certainly present in many nonthiosulfate-oxidizing *Athiorhodaceae* (Table 1). As growth in a cyanide-containing medium doubles rhodanese activity in *R. palustris* (S. Siskind, M. S. Thesis, The Pennsylvania State Univ., 1969), rhodanese may function in cyanide detoxification (4) by *Athiorhodaceae*.

We thank D. I. Arnon for the cell material of several strains of bacteria and for the assay facilities. Authorized for publication as paper no. 3803 in the journal series of the Pennsylvania Agricultural Experiment Station.

LITERATURE CITED

1. Bose, S. K. 1963. Media for anaerobic growth of photosynthetic bacteria, p. 501-510. *In* H. Gest, A. San Pietro, and L. P. Vernon (ed.), Bacterial photosynthesis. The Antioch Press, Yellow Springs, Ohio.
2. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
3. Rolls, J. P., and E. S. Lindstrom. 1967. Induction of a thiosulfate-oxidizing enzyme in *Rhodopseudomonas palustris*. J. Bacteriol. **94**:784-785.
4. Roy, A. B., and R. A. Trudinger. 1970. The biochemistry of inorganic compounds of sulphur. Cambridge Univ. Press, Cambridge, England.
5. Smith, A. J. 1964. Sulfur metabolism of *Chromatium* strain D and rhodanese activity in extracts. J. Gen. Microbiol. **34**:IX-X.
6. Smith, A. J., and J. Lascelles. 1966. Thiosulfate metabolism and rhodanese in *Chromatium* sp. strain D. J. Gen. Microbiol. **42**:357-370.
7. Truper, H. G., and H. D. Peck, Jr. 1970. Formation of adenylyl sulfate in phototrophic bacteria. Arch. Mikrobiol. **73**:125-142.