Enzymes of the reductive pentose phosphate cycle in the purple and in the green photosynthetic sulphur bacteria

The reductive pentose phosphate cycle has been proposed[1-3] as the main pathway of CO_2 fixation and its subsequent conversion to carbohydrate during photosynthesis. The fixation of CO_2 leads directly to the formation of 3-phosphoglycerate. The latter is partly converted to carbohydrate and partly utilized to regenerate the CO_2-acceptor. Recently, PETERKOFSKY AND RACKER[4] demonstrated that cell-free extracts of three photosynthetic species contained all the enzymes necessary for the operation of this cycle. However, a few of the enzymes were present in amounts judged insufficient to account fully for the rates of photosynthesis shown by intact cells. The authors suggested that other enzymes may be involved in individual steps of the cycle. Experiments conducted with the leaves of certain higher plants, notably soybean[5,6], and with the blue-green alga *Anacystis nidulans*[7-9] have indicated that variations in the reductive pentose phosphate cycle or alternative pathways for CO_2 fixation may exist in different photosynthetic organisms.

Although the photosynthetic bacteria have been widely used for research on photosynthesis, little is known about the pathway of CO_2 fixation and utilization in those bacteria capable of light-dependent autotrophic growth. Because of this and the variation in the metabolism of CO_2 which may occur in different photosynthetic organisms, cell-free extracts from two photosynthetic sulphur bacteria were examined for the requisite enzymes of the reductive pentose phosphate cycle.

Chromatium, strain D, a purple sulphur anaerobe, was cultivated at 35° on Hendley's medium[10]. The green sulphur anaerobe, *Chlorobium thiosulfatophilum*, strain L, was grown as described by LARSEN[11].

Bacterial extracts were prepared with the aid of a Raytheon 10 kc oscillator or a modified Hughes press[12]. The extracts in 0.01 *M* Tris buffer (pH 7.5) were centrifuged at 144000 × *g* for 90 min. The protein contents of the clear supernatant fluids were determined by the biuret reaction[13].

All assays were performed at 23°. Activities involving pyridine nucleotide oxidation or reduction were measured by continually recording the progress of the reaction using a Cary recording spectrophotometer, Model 14. Assays for most of the enzymes measured have been described previously[14] and assays for the remaining enzymes are given below. Transketolase was assayed as described by DE LA HABA *et al.*[15]. The reaction mixture in 1 ml contained bacterial extract, glycylglycine buffer (pH 7.5, 25 μmoles), $MgCl_2$ (3 μmoles), thiamine pyrophosphate (50 μg), mixed crystals of triose phosphate isomerase and α-glycerophosphate dehydrogenase (20 μg), isomerase product[15] (test cuvette only) and DPNH (0.1 μmole). Transaldolase was assayed by measuring glyceraldehyde 3-phosphate formation from a mixture of fructose 6-phosphate and erythrose 4-phosphate[16]. The reaction mixture in 1 ml contained bacterial extract, glycylglycine buffer (pH 7.5, 25 μmoles), mixed crystals of triose phosphate isomerase and α-glycerophosphate dehydrogenase (20 μg), fructose 6-phosphate (2 μmoles) D-erythrose 4-phosphate (kindly supplied by Dr. C. E. BALLOU) (1 μmole, test cuvette only), and DPNH (0.1 μmole). A correction was made for a slow reaction which occurred in the absence of added fructose 6-phosphate. The latter was prepared free of fructose 1,6-diphosphate by treatment with purified spinach fructose 1,6-diphosphatase[17]. Phosphopentoisomerase was assayed as described by

AXELROD[18] utilizing the cysteine–carbazole reaction for ketoses. The reaction mixture in 0.6 ml contained bacterial extract, Tris buffer (pH 7.0, 50 μmoles) and sodium ribose 5-phosphate (2 μmoles). Phosphoketopentoepimerase was assayed by following the production of xylulose 5-phosphate from ribose 5-phosphate and excess phosphopentoisomerase. The latter was purified according to TABACHNICK *et al.*[19]. The assay mixture in 0.5 ml contained bacterial extract, Tris buffer (pH 7.5, 50 μmoles), and excess phosphopentoisomerase. Controls containing the bacterial extract alone, and the ribose phosphate plus phosphopentoisomerase were included. Xylulose was measured as described by STUMPF AND HORECKER[20] after treatment of the deproteinized reaction mixture with 10 μg of purified *E. coli* alkaline phosphatase (Worthington Biochemical Corp.). This assay is not specific and the activities obtained should be considered as approximations.

Table I shows the results of a survey for enzymes of the reductive pentose phosphate cycle in cell-free extracts of *Chromatium* and *Chlorobium*. The extracts were also tested for phosphoglucoisomerase and phosphoglucomutase. Some of the results are taken from previous studies and the relevant publication is indicated in the table. Sedoheptulose 1,7-diphosphatase was not assayed. In combination with aldolase this enzyme could act as a by-pass for the transaldolase reaction[1].

Extracts from both bacteria contained all the enzymes which were assayed. The activities of the *Chromatium* enzymes were comparable with those found in other photosynthetic cells such as pea leaves and *Euglena gracilis* (SMILLIE, unpublished). In general, the activities of the *Chlorobium* enzymes were lower. This was not unexpected since the cells were grown at a lower temperature than *Chromatium* and their rate of growth and presumably rate of CO_2 fixation was considerably slower. However, some of the activities such as ribulose 1,5-diphosphate carboxylase and phosphopentoisomerase were especially low. Lack of data on the rates of photosynthesis by *Chromatium* and *Chlorobium* under the growth conditions employed precluded a

TABLE I

ENZYMES OF THE REDUCTIVE PENTOSE PHOSPHATE CYCLE IN CELL-FREE EXTRACTS FROM *Chromatium* AND *Chlorobium*

Enzyme	*Enzyme activity (μmoles/min/g protein)*	
	Chromatium	*Chlorobium*
Ribulose 1,5-diphosphate carboxylase	138*	12
3-Phosphoglycerate kinase	6020*	1160
Glyceraldehyde 3-phosphate dehydrogenase (DPN)	1830**	114**
Triose phosphate isomerase	4320*	405
Fructose 1,6-diphosphate aldolase	180*	48
Alkaline fructose 1,6-diphosphatase	228*	18
Phosphohexoisomerase	850*	590
Phosphoglucomutase	58*	25
Transketolase	280	92
Transaldolase	111	45
Phosphopentoisomerase	620	7
Phosphoketopentoepimerase	290	81
Ribulokinase	3000*	660

* See ref. 14.
** See ref. 21.

comparison of the enzymatic activities of the extracts with CO_2 fixation by the intact cells. In their studies with spinach leaves, *Euglena*, and *Chlorella*, PETERKOFSKY AND RACKER[1] noted that transaldolase, ribulose 1,5-diphosphate carboxylase (spinach excepting), fructose 1,6-diphosphatase (assayed at pH 7.4 to 7.8), and sedoheptulose 1,7-diphosphatase activities appeared to be insufficient to account for the rate of CO_2 fixation. Compared with the majority of the enzymes, transaldolase activity in the bacteria was not especially low, but this may not have been true for the carboxylase. Fructosediphosphatase activity from *Chromatium* was almost zero at neutral pH, but was high at pH 9.0. A fructose diphosphatase which is active at an alkaline pH rather than phosphatases acting at neutral or acid pH appears to participate in higher plant photosynthesis[22].

These results do not prove the operation of the reductive pentose phosphate cycle in the photosynthetic sulphur bacteria. They do indicate, however, that the mechanism of CO_2 fixation and conversion to carbohydrate is similar to that in the chemoautotrophic bacteria belonging to the *Thiobacilli* (for review, see ref. 23) and in higher plants such as spinach and pea. This conclusion is in agreement with experiments[14,24] which showed 3-phosphoglycerate to be one of the first stable products of CO_2 fixation in *Chromatium* cells.

This investigation was carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

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Received August 21st, 1961