Ribulose-1,5-Diphosphate Carboxylase of Rhodopseudomonas spheroides*

by

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The key role of ribulose-1,5-diphosphate (RuDP)-carboxylase in the reductive pentose phosphate cycle in autotrophic organisms of higher and lower form of plants has been well established (1, 2, 3). In a study of the phylogenetic distribution of RuDP-carboxylase, Fuller and Gibbs (4) found that the enzyme was also detectable in extracts of the heterotrophically grown cells of photosynthetic bacteria, Rhodospirillum rubrum and Chromatium sp. In a study of the inducible formation of RuDP-carboxylase in Rhodopseudomonas spheroides, Lascelles (5) demonstrated that light and anaerobic conditions were favorable for enzyme synthesis and that oxygen repressed enzyme formation completely. Recently Anderson et al. (6) reported a partial purification (20-40 fold purification) of RuDP-carboxylase from Rhodospirillum rubrum. The molecular weight of the enzyme was found to be small (M. W., 120,000; $S_{20,w}$ = 5.6S) compared with the enzyme of the plant origin (M. W., 515,000; $S_{20,w}$ = 17-18S)(7). Kinetic constants, Km ($NaHCO_3$, RuDP), were quite similar for bacterial and plant enzymes. Magnesium ions, however, do not appear to be essential for the carboxylation reaction with the bacterial enzyme (8).

Our recent experiments on the kinetic analyses of spinach leaf RuDP-carboxylase have established a strong homotropic effect with respect to CO_2 and cooperativity between CO_2 and Mg^{++} molecules (9, 10).

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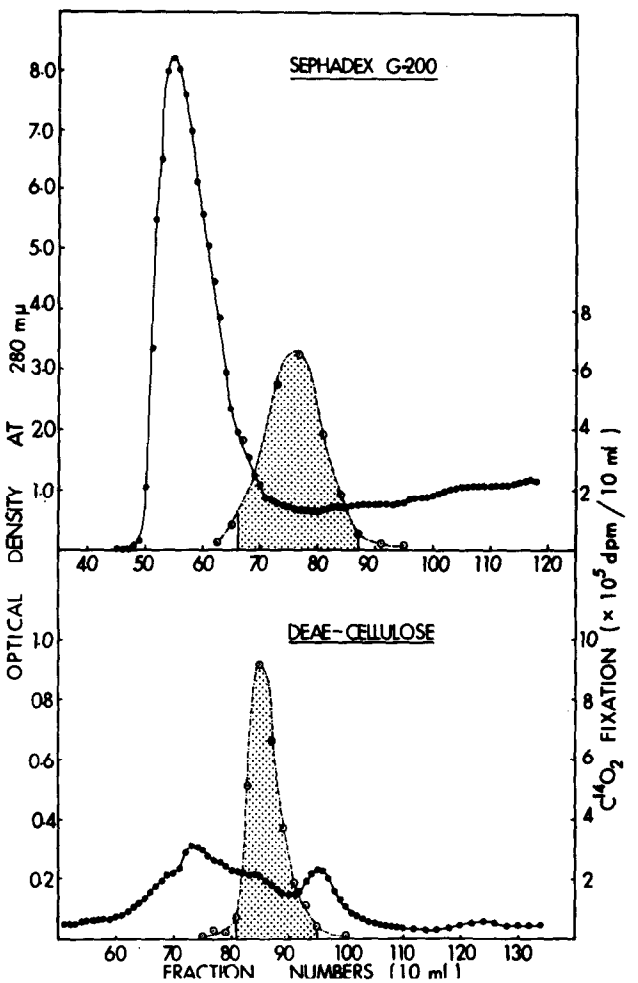

Fig. 1. Purification of RuDP-carboxylase of R. spheroides by Sephadex G-200 gel filtration (upper) and DEAE-cellulose column chromatography (lower).

Bacterial cell suspension (fresh wt., 30 g) was sonicated with a Raytheon-type ultrasonicator and centrifuged at 30,000 r.p.m. for 90 minutes. The supernatant fraction was subjected to an $(NH_4)_2SO_4$ precipitation at 50% saturation, and the precipitate collected was dissolved in 20 ml of 0.025 M Tris buffer (pH 7.5) containing 0.1 mM EDTA and 5 mM β-mercaptoethanol (Buffer A). After desalting by passage in a Sephadex G-25 column, 70 ml of eluate (containing about 1.4 g protein) was applied to an upward-flow column (5 × 85 cm) of Sephadex G-200, which was preequilibrated with Buffer A. Ten ml fractions were collected and 0.1 ml aliquots were used for the enzyme assay as described below. Eluate (tube Nos. 66-87, shadowed) was collected and subjected to an $(NH_4)_2SO_4$ precipitation again at 50% saturation. The precipitate was dissolved in 20 ml of Buffer A and after desalting in a Sephadex G-25 column; 30 ml eluate was charged on a DEAE-cellulose column (4 × 32 cm), equilibrated with Buffer A. A linear gradient of NaCl (0 - 1.0 M) was employed and 10 ml fractions were collected, and 0.1 ml aliquots were used for the enzyme assay. Contents of tube Nos. 81-95 (shadowed) were

collected and concentrated with Carbowax 6000 powder. The enzyme preparation obtained through a passage in a Sephadex G-25 column was used for the subsequent study.

The compositions of the reaction mixture were (in μmoles); Tris buffer (pH 7.8), 100; RuDP, 0.35; $NaHC^{14}O_3$, 25 (2.0 μc); $MgCl_2$, 2.5; and 0.1 ml of the enzyme preparation in a total volume of 0.5 ml. Incubation was at 25° for 10 minutes. For experimental details of the radioactivity measurements, see previous reports (9, 10).

In these respects, therefore, RuDP-carboxylase can be defined as a kind of allosteric enzyme. In view of the fact that there is a possibility that the role of Mg^{++} is to bind together subunits of the plant enzyme to form the catalytically active 18S oligomeric form, we attempted to determine the kinetic behavior of the bacterial RuDP-carboxylase, with particular emphasis on the role of Mg^{++} in the enzyme reaction.

A strain of R. spheroides provided by Professor G. Kikuchi was used throughout the experiments, using the chemically defined bacterial growth medium containing Na-glutamate and malate supplemented with minerals (11). The bacterial cells grown in the light and anaerobic conditions were harvested at the exponential growth stage. A crude extract obtained by the sonication was subjected to purification, employing (a) $(NH_4)_2SO_4$ precipitation at 50% saturation, (b) Sephadex G-200 gel filtration, and (c) DEAE-cellulose column chromatography (Fig.1). Using the standard enzyme assay system, about 20-fold purification was attained. The elution pattern from the Sephadex column presented in the figure indicates that the molecular weight of the bacterial enzyme was smaller than that of the plant RuDP-carboxylase. This result supports the previous observation of Anderson et al. with the R. rubrum enzyme (6).

Experimental results presented in Fig. 2A show that the reaction rate of the R. spheroides carboxylase is second order with respect to $NaHCO_3$ concentrations, similar to the case of the spinach enzyme (9,10). However, the addition of Mg^{++} affected neither the reaction rate nor the Vmax values. In both cases, n* values, calculated by the empirical Hill equation, were 2. A more obvious distinction between the plant and bacterial enzymes lies in the kinetic order of the latter enzyme with respect to RuDP. The rate of the $C^{14}O_2$ fixation as a function of the RuDP concentrations was examined at 3 different pH values in the absence of Mg^{++}. The sigmoidal curves portrayed in Fig. 3 clearly show the deviation of the reaction from Michaelis-Menten kinetics, the rate of $C^{14}O_2$ fixation being maximal in the neutral range. The addition of Mg^{++}, however, greatly stimulated the carboxylation reaction, normal-

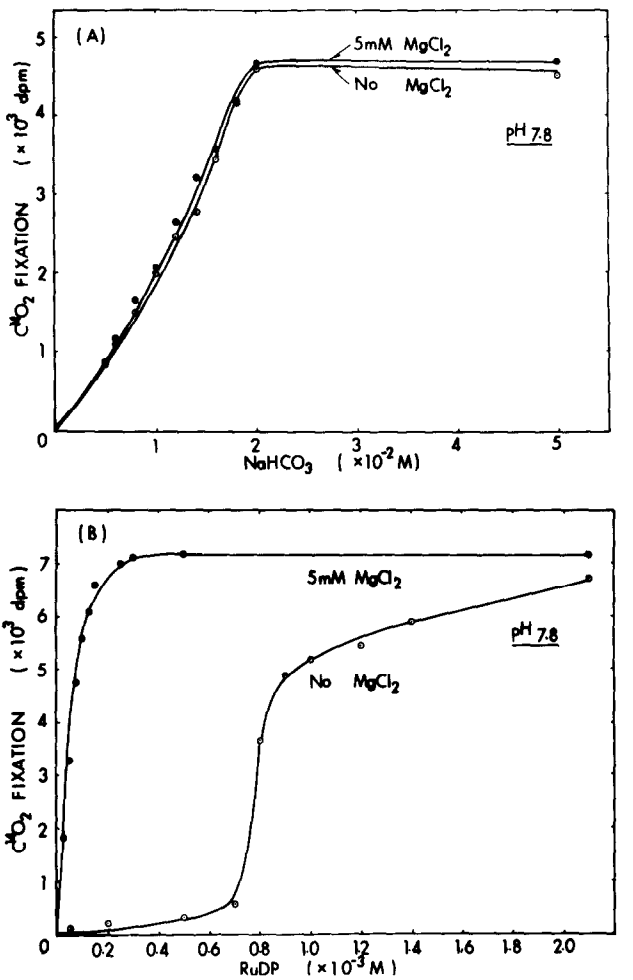

Fig. 2. Effect of Mg^{++} on reaction kinetics of R. spheroides RuDP-carboxylase.

A. Reaction rate ($C^{14}O_2$ fixation) vs. $NaHCO_3$ concentrations.

The compositions of the reaction mixture were (in μmoles): Tris buffer (pH 7.8), 100; RuDP, 1.05; $NaHC^{14}O_3$, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 25 (2.0 μc); $MgCl_2$, 0 or 2.5; and 0.1 ml of the enzyme preparation containing 6.8 μg N in a total volume of 0.5 ml. Incubation was at 25° for 15 minutes.

B. Reaction rate ($C^{14}O_2$ fixation) vs. RuDP concentrations.

The compositions of the reactions mixture were the same as those employed in experiments shown above, except 25 μmoles (2.0 μc) of $NaHC^{14}O_3$ and different quantities of RuDP (0.05, 0.10, 0.25, 0.35, 0.40, 0.45, 0.50, 0.60, 0.70, 1.05 μmole), were added to the reaction mixture.

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izing the substrate saturation curve to the Michaelian type, while the Vmax values remained unchanged (Fig. 2B). It is evident from the

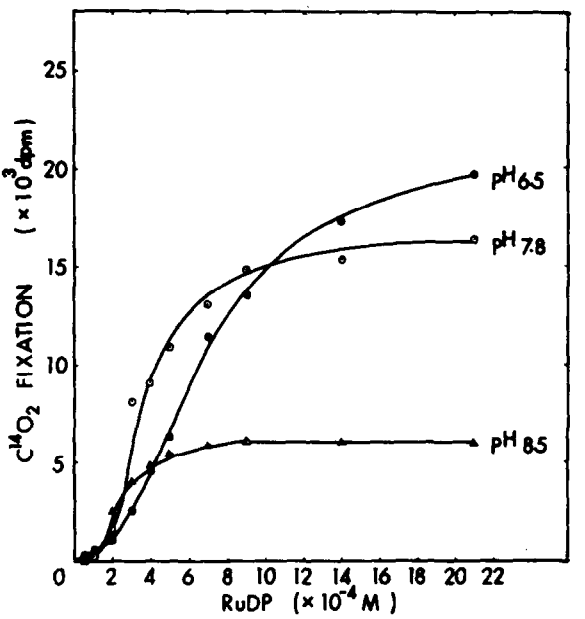

Fig. 3. Reaction kinetics ($C^{14}O_2$ fixation vs. RuDP concentrations) of R. spheroides RuDP-carboxylase at 3 different pH values in the absence of Mg^{++}.

The compositions of the reaction mixture were the same as those in Fig. 2, except different quantities of RuDP (0.025, 0.05, 0.10, 0.15, 0.20, 0.25, 0.35, 0.45, 0.70, 1.05 µmole), were added to the reaction mixture. The enzyme protein (0.1 ml) contained 13.3 µg N. Incubation was at 25° for 15 minutes.

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strong cooperativity between RuDP and Mg^{++} as shown in the figure that the apparent Km value of the bacterial enzyme toward RuDP declines markedly due to the Mg^{++}-binding.

The basis for the discrepancies between our finding of an absolute requirement for Mg^{++} in the RuDP-carboxylase reaction of R. spheroides and that of Anderson et al. indicating a lack of requirement for this cation with the R. rubrum enzyme is not known. However, one interpretation is that the latter workers might have employed experimental conditions for the maximum enzyme velocities in which Mg^{++} is not an activator.

Although the role of RuDP-carboxylase in the metabolism of the heterotrophically grown Athiorhodaceae is not clear, results of isotope experiments have suggested that RuDP-carboxylase may function normally in the carbon assimilation of the bacteria growing anaerobically (5). Experiments of Lascelles (5) have further shown that there exists a pos-

sible close connection between the regulation of the bacteriochlorophyll synthesis and the physiological function of RuDP-carboxylase. It appears evident that the concentration of RuDP is the decisive factor for photosynthetic CO_2-assimilation reactions in the bacteria growing heterotrophically, and that the level of Mg^{++} concentrations controls the enzymic carboxylation cooperatively with respect to RuDP. It will be an intriguing subject for future investigations to elucidate the molecular mechanism of the bacterial enzyme, which is so distinct from that of plant enzyme. Moreover, the comparison of the different enzyme kinetics between two types at the structure-function basis may provide a clue to the evolutional development of RuDP-carboxylase from lower microorganisms to higher autotrophic organisms.

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