PURIFICATION AND PURINE NUCLEOTIDE REGULATION OF RIBULOSE-1, 5-DIPHOSPHATE CARBOXYLASE FROM *THIOBACILLUS NOVELLUS*

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1. Introduction

The facultative autotroph *Thiobacillus novellus* was previously shown by the indirect method of $^{14}CO_2$ incorporation to possess ribulose-1, 5-diphosphate (RuDP) carboxylase (EC 4.1.1.39) activity when grown autotrophically [1]. However, when grown on organic compounds this activity was abolished. The present investigations were undertaken in order to directly show the presence of the enzyme and some of the methods of its regulation.

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2. Methods

A homogeneous preparation of RuDP carboxylase devoid of phosphoribulokinase activity was obtained as shown in table 1 by using a slight modification of the sucrose density gradient procedure of Goldwaite and Bogorad [2].

The preparation was found to be homogeneous by polyacrylamide gel 'disc' electrophoresis. Similarly, homogeneity was observed using both ultraviolet and Schlieren optical patterns during sedimentation velocity determinations. Schlieren patterns are shown in fig. 1. By these latter methods, an $S_{20,w}$ of 18.47 was obtained, this correlates fairly well with an average

Table 1

Purification of RuDP carboxylase from *T. novellus*. The spectrophotometric assay procedure of Hurlbert and Lascelles [13] was used. The reaction mixture contained: 80 mM Tris–HCl buffer (pH 8.2); 10 mM $MgCl_2$; 25 mM $NaHCO_3$; 0.2 mM RuDP; 30 μg purified enzyme protein and H_2O to a final volume of 1.0 ml. The reaction was stopped after incubating at 37°C for 5 min by boiling for 2 min. The 3-PGA present was determined in the following reaction mixture by following the ΔA_{340}nm. 25 mM Tris–HCl buffer (pH 8.2); 2.5 mM $MgCl_2$; 3.0 mM cysteine; 0.5 mM ATP; 0.125 mM NADH; 20 μl Sigma enzyme mixture GAPDH-PGK; 0.4 ml sample and water to a final volume of 1.0 ml. Each result represents the average of three determinations.

Sample	Total Protein (mg)	Total Activity (μmoles 3PGA/5 min)	Specific Activity (μmoles 3PGA/5 min/mg protein)	Purification (fold)	Percent Recovery
Crude extract	1100	214.5	0.195	–	100
Spinco supernatant	910	212.0	0.233	1.2	98.8
$MnCl_2$ treatment	673.4	167.0	0.248	1.3	77.9
$AmSO_4$ 35–40%	37	124.7	3.37	17.3	58.1
Sucrose[a], $AmSO_4$ and Amicon Ultrafiltration	9.3	104.2	11.2	57.4	48.6

[a] The pooled sucrose gradient fractions were saturated 40% with $(NH_4)_2SO_4$. The precipitated enzyme was centrifuged, resuspended in 0.2 M phosphate buffer (pH 7.8) and ultrafiltered on an Amicon XM-100A filter.

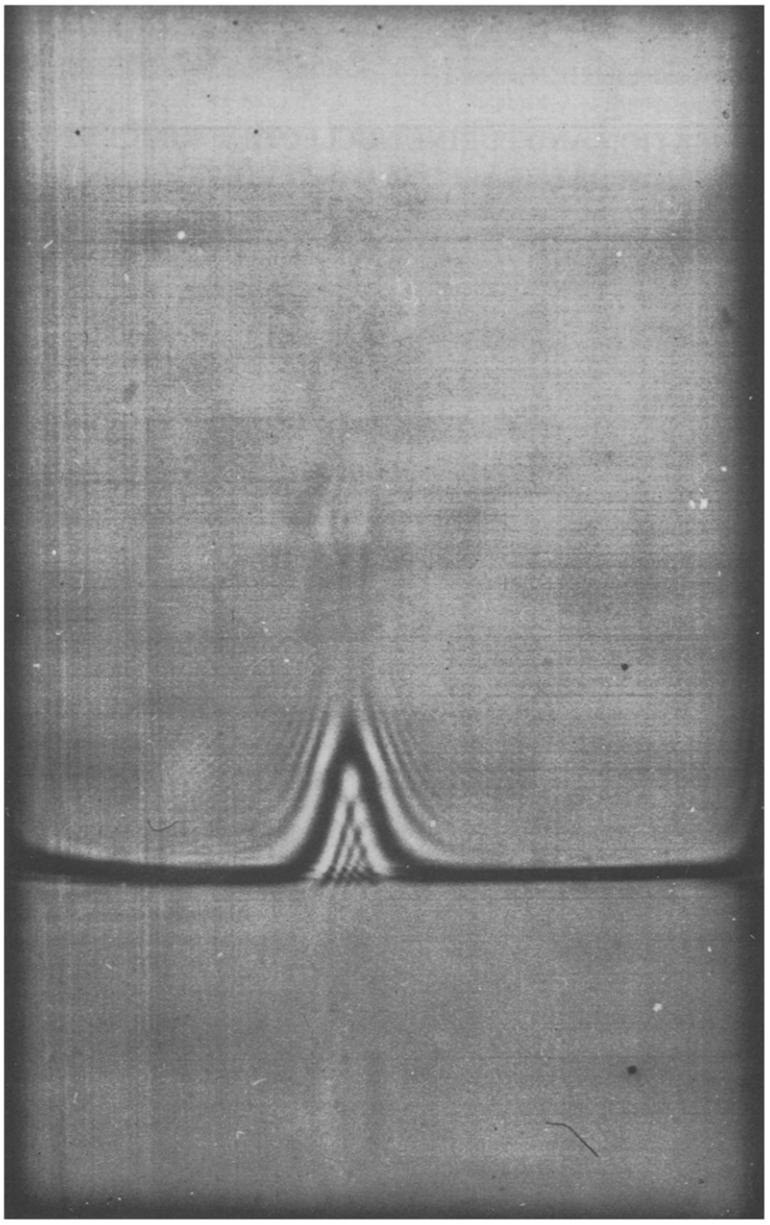

Fig. 1. Schlieren pattern of RuDP carboxylase. Picture was taken in a Beckman Model E analytical ultracentrifuge at 20°C and 52 000 rpm with a bar angle of 50°, 36 min after starting.

mol. wt. of 496 000 obtained from linear sucrose gradient centrifugations with alcohol dehydrogenase (mol. wt. 150 000) and catalase (mol. wt. 250 000) as standards.

The molecular size of the enzyme appears to be similar to that of enzymes isolated from *Thiobacillus thioparus* and *Thiobacillus neopolitanus* (17 S) [3], *Hydrogenomonas eutropha* (mol. wt. 515 000) and *Hydrogenomonas facilis* (mol. wt. 551 000) [4] and *Chromatium* strain D (mol. wt. 550 000) [5], as well as from various plant sources (6–8). However it is significantly larger than the enzyme isolated from

Table 2
Effect of purine and pyrimidine nucleotides on RuDP carboxylase.

Compound	Concentration (mM)	Specific Activity (μmoles 3PGA/5 min/mg protein)	Percent Activity
None	-	15.93	100
ATP	1.0	10.60	67
	0.1	12.12	76
ADP	1.0	4.54	29
	0.1	9.03	57
AMP	1.0	12.0	75
	0.1	12.8	80
GDP	1.0	8.37	53
	0.1	15.38	97
IDP	1.0	8.98	56
	0.1	15.14	95

The reaction mixture was essentially that outlined in table 1 except for the addition of the nucleotide. Results represent the average of three determinations.

Rhodopseudomonas palustris and *Rhodopseudomonas spheroides* (mol. wt. = 360 000) [9] and the strict autotroph *Thiobacillus denitrificans* (mol. wt. 365 000) [10]. If indeed increasing molecular weight of enzymes is indicative of a later evolutionary development as has been suggested [4], *T. novellus* may be a representative of a later form of autotrophy than some thiobacilli and purple, non-sulfur photosynthetic bacteria.

Table 2 shows the effect of purine and pyrimidine nucleotides on enzyme activity. The adenine nucleotides, especially ADP, were the most inhibitory although IDP and GDP were also inhibitory to a similar but lesser extent. The order of inhibition among the adenine nucleotides was ADP>ATP>AMP.

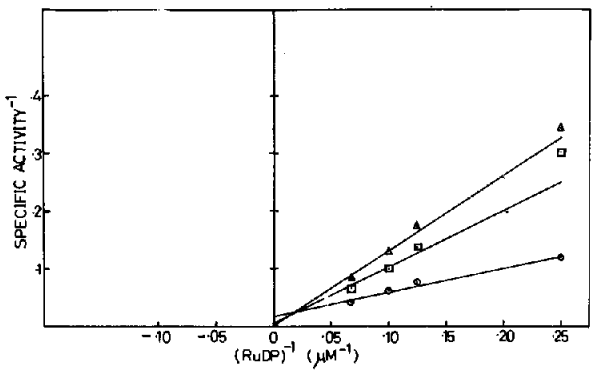

Fig. 2. Double reciprocal plots of the effect of ADP vs varying RuDP concentration. (○—○—○) no ADP; (□—□—□) 0.3 mM ADP; (△—△—△) 0.6 mM ADP.

Double reciprocal plots of the effect of ADP against varying RuDP concentration is shown in fig. 2.

At a concentration of 0.1 or 1.0 mM, there was no significant effect on enzyme activity in the presence of GTP, GMP, ITP, IMP, TTP, TDP, TMP, UTP, UDP, UMP, CTP, CMP, PP_i, adenosine, cytidine, guanosine, uridine, adenosine tetraphosphate, 3′, 5′ cyclic AMP, adenine sulphate, adenine phosphate or adenosine-5′-diphosphoribose. Similarly, NAD^+, NADH, $NADP^+$ or NADPH showed no significant effect at 0.1 mM.

The high levels of inhibition (43%) obtained with low concentrations (0.1 mM) of ADP seem to be indicative of a regulatory role for this compound during CO_2 fixation by RuDP carboxylase. Structurally it is unlike RuDP. Since neither adenosine, adenine nor pyrophosphate had effect on enzyme activity, and since either IDP or GDP were equally inhibitory, albeit to a lesser degree, it seems that the presence of a group at the 6′ position of the adenine nucleus together with at least one phosphate group at the 5′ position of ribose is essential for inhibition.

At present the results do not show whether the inhibition observed is due to allosteric transition or steric hindrance [11]. Further studies are needed to elucidate this point.

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