INHIBITION OF RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE BY SEDOHEPTULOSE-1,7-BISPHOSPHATE

Ashok K. SALUJA and Bruce A. McFADDEN

Program in Biochemistry and Biophysics, Department of Chemistry, Washington State University, Pullman, WA 99164, USA

Received 6 October 1978

1. Introduction

D-Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (3-phospho-D-glycerate carboxylase (dimerizing) EC 4.1.1.39) is a key catalyst of the Calvin cycle and catalyzes the reaction of RuBP with carbon dioxide to give two molecules of 3-phosphoglycerate or with oxygen to give one 3-phosphoglycerate and one phosphoglycolate in most if not all autotrophs [1–3]. These two reactions represent a branch point in carbon flow resulting in photosynthesis or photorespiration (discussed [4]), and it seems likely that the regulation of either or both activities may control the overall carbon metabolism in these organisms.

It is logical that one or more intermediate(s) of the Calvin cycle should regulate the activities of RuBP carboxylase/oxygenase. We describe here the inhibition of these activities in the barley enzyme by sedoheptulose-1,7-bisphosphate (SBP) and its possible role in regulating photosynthesis and photorespiration.

2. Materials and methods

2.1. *Materials*

Tetrasodium salts of RuBP, fructose-1,6-bisphosphate (FBP) and SBP (>91% pure) were obtained from Sigma Chemical Co. $NaH^{14}CO_3$ was purchased from Schwarz/Mann. Other chemicals used during this investigation were of a reagent quality.

2.2. *Enzyme purification*

To obtain the enzyme, seedlings of barley (*Hordeum vulgare* variety Steptoe) were grown in plastic racks for 7 days at 18 ± 1°C in continuous light (~370 lux) and at high humidity. A homogenate was prepared by grinding the leafy tissue in a chilled Waring blender. RuBP carboxylase/oxygenase was purified by the procedures developed by A.K.S. and B.A.McF. (unpublished) by precipitating the enzyme at 30–50% saturation with respect to $(NH_4)_2SO_4$ followed by sedimentation into a 0.2–0.8 M linear sucrose density gradient. Peak fractions were pooled and dialysed against TEMBD (pH 8.0) at 25°C (i.e., buffer containing 20 mM Tris, 1 mM EDTA, 20 mM $MgCl_2$, 50 mM $NaHCO_3$, unless otherwise specified, and 1 mM dithiothreitol). The final specific activity of the gel-electrophoretically homogeneous enzyme was 1.5 μmol CO_2 fixed/min/mg protein.

2.3. *RuBP carboxylase assay*

For the assay of RuBP carboxylase [5] the reaction mixture contained in 250 μl: 1.2 μmol $MgCl_2$, 0.4 μmol EDTA, 0.1 μmol dithiothreitol, 6 μmol $NaH^{14}CO_3$ (spec. act. 0.1 μCu/μmol), 2.0 μmol Tris–Cl (pH 8.0 at 25°C), 0.2 μmol RuBP and 10–40 μg enzyme. The reaction mixture (except RuBP which was contained in 50 μl) was preincubated for at least 5 min at 30°C before initiating the reaction at 30°C with RuBP. The reaction was terminated with 60% cold trichloroacetic acid 1 min after the addition of RuBP. An aliquot of 200 μl was transferred to a scintillation vial and excess $^{14}CO_2$ was liberated at 70–80°C for 1 h prior to radioactive counting. The activity was measured as RuBP-dependent incorporation of $^{14}CO_2$ into acid-stable product.

2.4. *RuBP oxygenase assay*

The oxygenase activity was monitored at 30°C by measuring RuBP-dependent oxygen consumption using a oxygen electrode (Hansatech Ltd) with a continuous recording of oxygen uptake. The reaction mixture (in a 0.75 ml chamber) consisted of 'CO_2-free' buffer containing 50 mM Tris–Cl, pH 8.6 (25°C), 10 mM $MgCl_2$, 1 mM EDTA, 1.3 mM $NaHCO_3$, the enzyme, and 1 mM RuBP unless otherwise specified. The reaction (in air) was initiated with enzyme which had been preincubated at 30°C for 40 min in TEMB containing 20 mM $NaHCO_3$ and the initial linear time course of oxygen consumption was used to calculate the reaction velocity. Protein was determined by the Lowry method [6].

3. Results

3.1. *Inhibition of carboxylase*

SBP, at low concentrations was a very potent linear competitive inhibitor [7] with respect to RuBP (fig.1). The K_i for this inhibition was 75 μM which was less than the K_m (100 μM) for RuBP for carboxylase activity.

The extent of inhibition of the carboxylase activity by SBP was independent of the time of preincubation of the enzyme (at 30°C) with the inhibitor. However, the activity of the enzyme increased by 50% in 45 min upon incubation at 30°C. The rate of CO_2 fixed (both with and without inhibitor) was constant for at least 3 min and the % inhibition of activity was independent of the assay time.

Sedoheptulose-7-phosphate, another Calvin-cycle intermediate, did not significantly inhibit the carboxylase activity at 1 mM when added at zero time with RuBP.

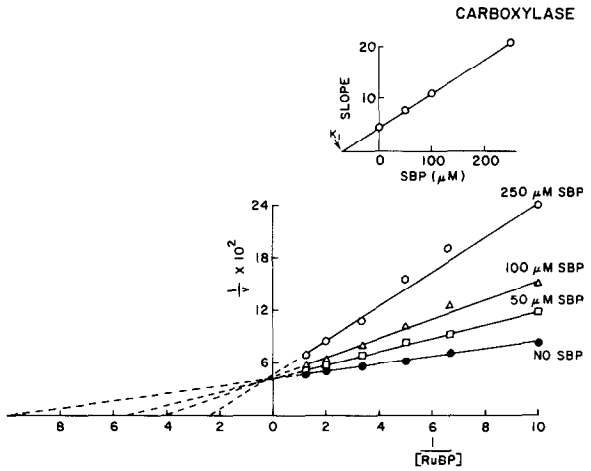

Fig.1. Double reciprocal plot of RuBP carboxylase activity versus RuBP concentration in the absence and presence of the indicated concentrations of SBP. The enzyme activity is expressed as nmol CO_2 fixed/min and RuBP concentrations are in mM units. Enzyme, 15 μg, was used in each assay and the reaction mixture (including SBP) was preincubated as described in section 2. The 1 min assay was initiated by adding RuBP. The lines were generated by linear least square analysis. The V_{max} values were within ± 5%. Insert: the slopes of these lines are plotted against the respective SBP concentration.

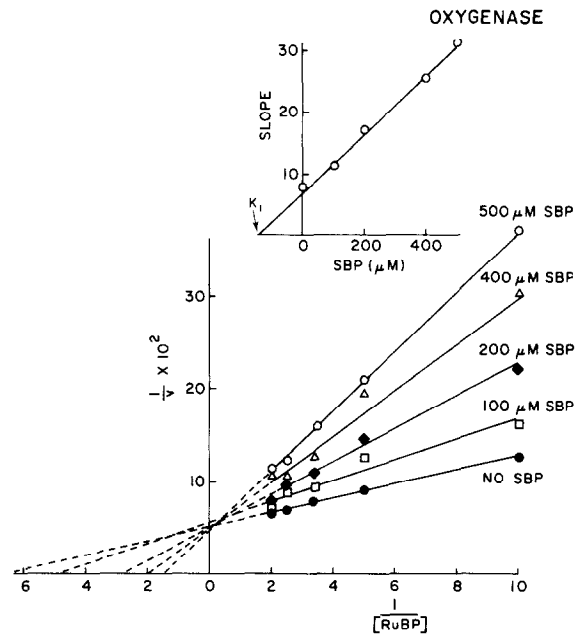

Fig.2. Double reciprocal plot of RuBP oxygenase activity versus RuBP concentration in the absence and presence of the indicated concentrations of SBP. The enzyme activity is expressed as nmol oxygen consumed/min and RuBP concentrations are in mM units. The activated 150 μg enzyme detailed in section 2 was used in each assay (spec. act. 0.1 μmol RuBP-dependent oxygen consumption/min/mg protein). SBP was added with RuBP and the reaction was initiated by adding the enzyme. The enzyme activity was calculated from the slope of the initial time course of the oxygen consumption. The lines were generated by linear least square analysis and the V_{max} values were within ± 5%. Insert: the slopes of these lines are plotted against the respective SBP concentration.

3.2. *Inhibition of oxygenase*

The oxygenase activity was also inhibited by SBP in a manner that was linearly competitive [7] with respect to RuBP (fig.2). The data yield a K_i value of 135 μM, which is slightly less than the K_m (150 μM) for RuBP for the oxygenase activity.

4. Discussion

The regulation of RuBP carboxylase may be of considerable importance in influencing CO_2 assimilation via the Calvin cycle in autotrophic organisms. Obviously, intermediates in this cycle may regulate RuBP carboxylase/oxygenase. However, the effect of these intermediates on enzyme activity is relatively unknown. Substantial stimulation of the carboxylase activity by fructose-6-phosphate (560%), ribulose-5-P (360%), xylulose-5-P (270%) and ribulose-5-P (120%), all at 0.5 mM has been reported [8] but we were unable to obtain significant stimulation of this activity by any of these compounds (at 2 mM) at 30°C by preincubation with properly activiated barley enzyme (A.K.S. and B.A.McF., unpublished data). These differences may be due to the fact that the enzyme was not fully activated [8]. Inhibition of the carboxylase activity by fructose-1,6-bisphosphate has also been observed [9] but the K_i is >5-fold higher than the K_m for RuBP.

We describe here the strong inhibition of both activities of RuBP carboxylase/oxygenase by sedoheptulose-1,7-bisphosphate (SBP), an intermediate of the Calvin cycle. Sedoheptulose-7-phosphate did not inhibit either of these activities, thereby indicating that inhibition is specifically due to SBP and not due to the monophosphate which may be a contaminant in SBP.

Recently it has been reported [10] that 6-phosphogluconate, which is a competitive inhibitor of pseudomonad RuBP carboxylase, enhances this activity when present at low concentrations during pre-incubation of the enzyme with sub-optimal levels of Mg^{2+} and HCO_3^-. These investigators have reiterated the importance of distinguishing between the action of effectors upon catalysis and/or activation [11]. Our results (data not shown) indicate that SBP inhibits only catalysis and has no effect on the activation of the enzyme. The similar mode of inhibition of both carboxylase and oxygenase activities indirectly reinforces the evidence that a single protein catalyzes both reactions.

Since the K_i values obtained for both activities are in range of the corresponding K_m values for RuBP and are less than the physiological concentration of SBP [12], it seems likely that the inhibition by SBP plays a significant role in controlling the activities of this enzyme in vivo and thus may regulate both photosynthesis and photorespiration.

Acknowledgements

We acknowledge support in part by grants from the Frasch Foundation and NIH (GM-19 972).

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