Influence of the Activation State on the Sedimentation Properties of Ribulose Bisphosphate Carboxylase from Alcaligenes eutrophus*

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Botho Bowien and Ellen-Marie Gottschalk§
From the Institut für Mikrobiologie and the §Zentrum für Biochemie der Universität Göttingen, D-3400 Göttingen, Federal Republic of Germany

Ribulose-1,5-bisphosphate carboxylase from the chemolithotrophic hydrogen bacterium Alcaligenes eutrophus was maximally active in the presence of 50 mM HCO$_3^-$ plus 10 mM Mg$^{2+}$. Deactivation occurred upon removal of these ions. Reactivation was achieved by incubation of the enzyme with HCO$_3^-$ plus Mg$^{2+}$. The concentration of HCO$_3^-$ (CO$_2$) required for half-maximal activation was 1.84 mM (0.064 mM). Sedimentation velocity studies revealed that activation/deactivation is associated with drastic changes in the sedimentation properties of the enzyme. While the inactive form had a sedimentation coefficient, $s_{20,w}$, of 17.5 S, the $s_{20,w}$ gradually decreased as the enzyme was reactivated and the fully reactivated form exhibited an $s_{20,w}$ of 14.3 S. A structural analogue of ribulose 1,5-bisphosphate, xylulose 1,5-bisphosphate, caused a deactivation of the enzyme concomitant with an increase in the sedimentation velocity. It is suggested that the alterations in the hydrodynamic properties accompanying the activation/deactivation process are due to considerable conformational changes that affect the molecular volume and/or the shape of the enzyme. Dissociation/association events were not involved in the changes. The $s_{20,w}$ of about 18 S, generally reported for the large hexadecameric ribulose bisphosphate carboxylases, appears to be characteristic of the inactive form.

The majority of photoautotrophic and chemoaotrophic organisms assimilates CO$_2$ through the reductive pentose phosphate cycle. Ribulosebisphosphate carboxylase (EC 4.1.1.39) is the key enzyme of this primary biosynthetic pathway. The enzyme also functions as a RuBP$^1$ oxygenase catalyzing the first step in the photosynthetic or chemorespiratory glycolate metabolism of autotrophs (1). RuBP carboxylase from plants and algae as well as bacteria is a large enzyme ($M_r = 500,000-550,000$) with a sedimentation coefficient, $s_{20,w}$, usually reported to be about 18 S. It is multimeric, consisting of eight large, catalytic subunits and eight small subunits of unknown functions (2). The enzyme of the chemolithotrophic hydrogen bacterium Alcaligenes eutrophus also has this quaternary structure (3-5). CO$_2$ plus Mg$^{2+}$ activate RuBP carboxylase by formation of a ternary enzyme-CO$_2$-Mg complex (6-8). The activation involves a carbamate formation at distinct activator sites identified as lysine 201 of the large subunit of the spinach enzyme (9-11). Conformational studies on the spinach and pea RuBP carboxylases using fluorescent (12, 13) and circular dichroism measurements (14) yielded indirect evidence for structural changes induced by CO$_2$ plus Mg$^{2+}$. However, nothing is known about the nature and extent of the conformational changes associated with the activation process of the enzyme. Sedimentation analysis is one suitable approach to study such alterations (15, 16). Previous investigations have shown that the RuBP carboxylase from A. eutrophus exhibited an unusually low $s_{20,w}$ of 14.1 S in a buffer system containing HCO$_3^-$ (CO$_2$) and Mg$^{2+}$ (4), i.e. under conditions which presumably supported the active state of the enzyme. In the present communication, we provide evidence for reversible changes in the sedimentation properties of A. eutrophus RuBP carboxylase. These changes were correlated with the activation state of the enzyme.

EXPERIMENTAL PROCEDURES

Materials—Homogeneous RuBP carboxylase from A. eutrophus strain H16 (ATCC 17699) was isolated as previously described (3). The maximal specific activity of the enzyme measured at pH 7.8 and 30 °C was 1.67 units·mg of protein$^{-1}$ (1 unit = 1 mmol of CO$_2$ fixed·min$^{-1}$). Protein contents of enzyme solutions were determined by absorbancy measurements at 280 nm as reported before (3). RuBP was obtained from Sigma. XuBP which had been prepared according to McCurry and Tolbert (17) was a generous gift from Dr. J. V. Schloss. NaHCO$_3$ came from The Radiochemical Center, Amersham, U. K.

Enzyme Deactivation/Reactivation and Assay—Active RuBP carboxylase dissolved in Tris buffer (20 mM Tris-HCl, pH 7.8, 50 mM NaHCO$_3$, 10 mM MgCl$_2$, 1 mM EDTA, 1 mM dithioerythritol) was deactivated by thorough dialysis against this buffer lacking added NaHCO$_3$ and MgCl$_2$. Subsequent reactivation was performed by incubating the enzyme (0.43-0.46 mg·ml$^{-1}$) in Tris buffer, containing various indicated concentrations of NaHCO$_3$ and MgCl$_2$ at 30 °C for 45 min. The activity assay was carried out as described earlier (3) except that the reaction was initiated with the enzyme and terminated 3 h later with 0.10 ml of 7 M phosphoric acid.

Ultracentrifugal Analyses—The sedimentation experiments were performed in a Beckman Spinco Model E ultracentrifuge equipped with monochromator, split beam photoelectric scanning absorption system, and multiplexer. Double sector cells with monochromator, split beam photoelectric scanning absorption system, and multiplexer. Double sector cells with 12-mm optical path and sapphire windows were used. In the sedimentation velocity studies, the RuBP carboxylase solutions, containing 0.43-0.46 mg·ml$^{-1}$ in Tris buffer supplemented with NaHCO$_3$ and MgCl$_2$ at the stated concentrations, were centrifuged at a speed of 36,000 rpm and a temperature of 5 °C. Scanner tracings at 280 nm were taken at 8-min intervals. Sedimentation coefficients were calculated by the moving boundary method (18) and corrected to values ($s_{20,w}$) corresponding to a solvent with the viscosity and density of water at 20 °C.

Sedimentation equilibrium centrifugations for molecular weight determinations were conducted by the low speed method of Van Holde and Baldwin (19). The same enzyme solutions that were used in the velocity runs were centrifuged at 4600 rpm and 5 °C for 36-40 h. A volume of 0.728 ml·g$^{-1}$ was employed for the partial specific volume, $\nu$, of the enzyme (4).

RESULTS AND DISCUSSION

Like all RuBP carboxylases so far studied, the A. eutrophus enzyme was deactivated upon removal of CO$_2$ and Mg$^{2+}$. The
residual activity of the deactivated (inactive) enzyme was about 6% (0.10 unit·mg⁻¹) of that measured for the fully active enzyme (1.87 unit·mg⁻¹). However, no attempt was made to completely eliminate the CO₂ present in the solutions at a concentration (9 μM) corresponding to its mixing ratio in air (0.03%, v/v). Reactivation was achieved by incubation of the enzyme with CO₂ plus Mg²⁺. Complete reactivation at 10 mM Mg²⁺ required the presence of 40-50 mM HCO₃⁻ (Fig. 1). For half-maximal reactivation, 1.84 mM HCO₃⁻ (0.064 mM CO₂) was necessary. The latter value resembles those obtained for spinach RuBP carboxylase (20, 21).

Sedimentation velocity studies were performed with the enzyme incubated at increasing HCO₃⁻ concentrations plus 10 mM MgCl₂. Substantial changes in the sedimentation properties associated with the activation state of the enzyme were evident (Fig. 1). The gradual conversion from the inactive into the most active form was accompanied with a decrease in the sedimentation coefficient from 17.5 S down to 14.3 S. Thus, the $s_{\text{ro,av}}$ of 14.1 S previously reported for the A. eutrophus carboxylase (4) is characteristic of the fully activated enzyme. A comparison of the integral sedimentation distributions confirmed the distinctly different sedimentation properties of inactive carboxylase (Fig. 2). No significant boundary spreading was observed in the sedimentations with enzyme at all tested states of activation, indicating that the solutions contained uniformly sedimenting enzyme molecules. The enzyme partially reactivated (35%) with 50 mM HCO₃⁻ alone showed an intermediate sedimentation coefficient of 15.1 S. In contrast, incubation of the carboxylase with 10 mM Mg²⁺ in the absence of added HCO₃⁻ did not result in reactivation and the $s_{\text{ro,av}}$ of the enzyme was accordingly high (17.4 S). The decrease of $s_{\text{ro,av}}$ was not due to unspecific effects of the increasing ionic strength of the buffer solutions. Preincubation and sedimentation of the enzyme in buffer containing 70 mM NaCl instead of 50 mM NaHCO₃ and 10 mM MgCl₂ gave an $s_{\text{ro,av}}$ of 17.5 S with the enzyme being inactive.

A survey of the literature revealed that the high sedimentation coefficients of about 18 S, generally reported for the large plant type RuBP carboxylases, were almost invariably obtained with the enzymes dissolved in buffer systems lacking added HCO₃⁻ and Mg²⁺, i.e. most probably with the inactive forms. When active carboxylases were analyzed like those from the hydrogen bacteria Paracoccus denitrificans (22, 23), Nocardia opaca, and Arthrobacter 11/x (24), the $s_{\text{ro,av}}$ values ranged from 14.0 S to 14.4 S. The reversible gross alterations in the sedimentation properties apparently did not involve dissociation/reassociation phenomena detectable by ultracentrifugal analysis. For both forms of the A. eutrophus enzyme, a molecular weight of $M_r = 535,000$ was determined by low speed sedimentation equilibrium centrifugation. Plots of the equilibrium data showing the dependence of log $c$ on $r^2$ is given. Initial enzyme concentrations were 0.255 mg·ml⁻¹ for the active (○) and 0.275 mg·ml⁻¹ for the inactive (●) enzyme. $c = $ enzyme concentration; $r = $ distance of sample from axis of rotation.

![Fig. 1. The effect of HCO₃⁻ (CO₂) concentration on the activation and sedimentation properties of RuBP carboxylase from A. eutrophus. Specific activity (○) and sedimentation coefficient, $s_{\text{ro,av}}$ (●), are plotted against the HCO₃⁻ concentration. The various Tris buffers used during the preincubation of the enzyme and the centrifugations contained the indicated NaHCO₃ concentrations plus 10 mM MgCl₂. See "Experimental Procedures" for further experimental details.](image1)

![Fig. 2. Integral sedimentation distribution of active and inactive RuBP carboxylase. The concentration of the enzyme, $c$, relative to the initial concentration, $c_0$ (0.43 mg·ml⁻¹), is plotted as a function of sedimentation coefficient, $s_{\text{ro,av}}$. Centrifugation of active enzyme (○) was in Tris buffer containing 50 mM NaHCO₃ plus 10 mM MgCl₂, that of inactive enzyme (●) in Tris buffer lacking added HCO₃⁻ and Mg²⁺. The apparent $s_{\text{ro,av}}$ values correspond to $c/c_0 = 0.5$.](image2)

![Fig. 3. Sedimentation equilibrium of active and inactive RuBP carboxylase. The dependence of log $c$ on $r^2$ is given. Initial enzyme concentrations were 0.255 mg·ml⁻¹ for the active (○) and 0.275 mg·ml⁻¹ for the inactive (●) enzyme. $c = $ enzyme concentration; $r = $ distance of sample from axis of rotation.](image3)
sedimentation of the enzyme and a concomitant increase of the sedimentation properties of RuBP carboxylase. The dimeric enzyme from \( A. eutrophus \) probably binds at the catalytic site of the enzyme and a concomitant increase of the sedimentation coefficient (Table 1). Incubation of active \( A. eutrophus \) carboxylase with XuBP caused a deactivation of the enzyme and a concomitant increase of the sedimentation coefficient (Table 1). If the binding of the actual substrate RuBP has a similar effect, the enzyme may only be partially activated in its presence. Indeed, it has been shown that the spinach enzyme activates very slowly if RuBP is present (20). Binding of RuBP to the probably inactive form of the spinach enzyme had no significant effect on the sedimentation properties (25).

The increase in sedimentation velocity of \( A. eutrophus \) RuBP carboxylase, occurring upon deactivation, amounted to maximally 23%. Such drastic alterations in the hydrodynamic properties of the enzyme necessarily reflect considerable conformational changes. These changes may not only be confined to the tertiary structure of the constituent subunits but could also involve a rearrangement of the enzyme’s quaternary structure. It is suggested that the activation by CO\(_2\) plus Mg\(^{2+}\) is associated with an increase of the molecular volume and/or a change in the shape (axial ratio) of the enzyme. However, the effective Stokes radius as determined by gel filtration chromatography remained constant at 6.1 nm, regardless of the activation state of the enzyme (data not shown). Small angle x-ray scattering studies which are presently being performed are expected to yield information on the type of changes. Preliminary sedimentation experiments with spinach carboxylase gave results compatible with those obtained for the bacterial enzyme. Therefore, the large, oligomeric RuBP carboxylases may undergo similar structural alterations during activation/deactivation. An interesting question concerns the involvement of the small subunits in the conformational changes. The dimeric enzyme from \( Rhodospirillum rubrum \) which lacks the small subunits (26) should thus also be subjected to sedimentational analysis.

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**REFERENCES**


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**Table 1**

*Effect of xylulose 1,5-bisphosphate on the activity and the sedimentation properties of RuBP carboxylase*

<table>
<thead>
<tr>
<th>Concentration of XuBP* (mM)</th>
<th>Specific activity</th>
<th>Sedimentation coefficient</th>
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<tbody>
<tr>
<td>0</td>
<td>1.67</td>
<td>14.2 S</td>
</tr>
<tr>
<td>0.01</td>
<td>1.24</td>
<td>16.8 S</td>
</tr>
<tr>
<td>0.1</td>
<td>1.07</td>
<td>16.8 S</td>
</tr>
<tr>
<td>1.0</td>
<td>0.57</td>
<td>17.7 S</td>
</tr>
</tbody>
</table>

* The compound is also a competitive inhibitor of the enzyme with respect to RuBP when added simultaneously with RuBP (15, and this paper).