THE DEACTIVATION AND REACTIVATION OF RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE AND OXYGENASE DURING AIR–ARGON–OXYGEN TRANSITIONS

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1. Introduction

Ribulose-1,5-bisphosphate (RuBP) carboxylase-oxygenase, preactivated with CO_2 and Mg^{2+}, is only active in the presence of oxygen. The removal of oxygen causes slow deactivation of both activities with similar kinetics. The readdition of oxygen reversibly activates the enzyme after a significant lag phase. The measurements of intrinsic protein fluorescence show that the deactivation and reactivation process is linked to a reversible conformational change of the enzyme molecules.

The enzyme pretreated with RuBP is not affected by oxygen removal. Furthermore, the addition of a sulfhydryl reducing reagent such as β-mercaptoethanol prevents the deactivation of the Mg^{2+}–CO_2-preactivated enzyme during anaerobic conditions.

2. Materials and methods

RuBP carboxylase-oxygenase was isolated from spinach leaves. The enzyme was purified to homogeneity by ammonium sulfate fractionation, Sephadex G-200 chromatography and a sucrose density gradient centrifugation step [1]. The fractions with the highest specific activities (1.15 μmol CO_2 fixed/mg protein. min; and 0.13 μmol O_2 consumed/mg protein.min, respectively) were pooled and used throughout all experiments.

The enzyme was dialysed against 50 mM Tris–sulfate buffer, (pH 8.0) with additions as indicated in table 1. The dialysed enzyme (14.8 mg protein in 4 ml) was gassed with argon or nitrogen in an oxygraph cell (Rank Brothers, Cambridge) with a gas flow of 0.5–1 l/h; the oxygen content of the cell was recorded. Samples were taken and preactivated for 15 min with 10 mM $MgCl_2$ and 10 mM $NaHCO_3$ in argon atmosphere and then assayed for RuBP carboxylase and oxygenase activity. The assay conditions for the carboxylase and the oxygenase reactions have been described [2].

At the end of the anaerobic treatment, the oxygraph cell was gassed with oxygen until the oxygen content of the enzyme solution reached ~ 0.5 mM; enzyme samples were taken, preactivated, and analysed again for RuBP carboxylase and oxygenase activity.

The intrinsic protein fluorescence was measured in a Zeiss spectrophotometer (PMQII-ZMF4-M4QIII) equipped with a second monochromator (M4QIII). The exciting light beam (Zeiss Xe-lamp LX501, 286 nm) passed through a 1 mm slit; the fluorescence emission was recorded at 340 nm. The enzyme was diluted 80-fold with the incubation buffer.

3. Results

When RuBP carboxylase-oxygenase was preactivated with Mg^{2+} and CO_2 and then incubated with argon, the enzyme was severely deactivated (fig.1).

The dialysed enzyme fraction was gassed with argon at 25°C. After 20 min the oxygen content of the cell dropped to 3 nmol oxygen/ml; the ratio of enzyme molecules to measurable oxygen was equivalent to ~ 2. The analysis of the enzyme activities showed that both RuBP carboxylase and oxygenase retained

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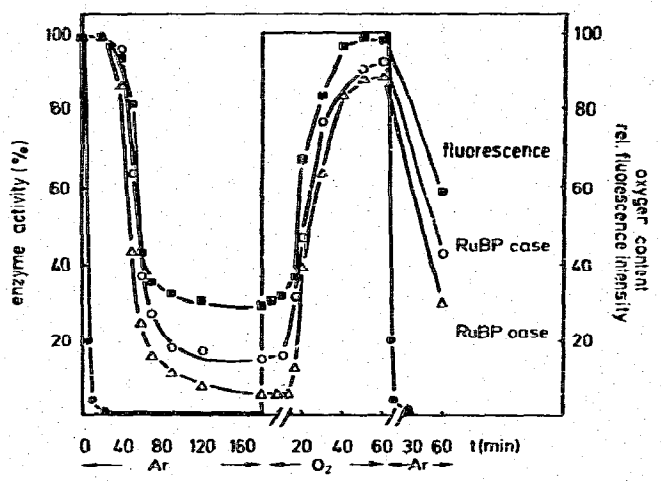

Fig.1. The time course of deactivation and reactivation of RuBP carboxylase–oxygenase. Preactivated enzyme (10 mM $MgCl_2$ + 10 mM $NaHCO_3$) was gassed with argon at 25°C. After 180 min exposure to argon the enzyme was oxygenated for 60 min and subsequently gassed with argon again. Aliquots were taken and analysed for RuBP carboxylase (○——○), RuBP oxygenase (△——△), and for intrinsic protein fluorescence (■——■); the oxygen content of the incubation cell was monitored polarographically (●——●).

full activity. Further gassing with argon caused a severe loss of both activities, which occurred with similar kinetics. This loss of enzyme activities was not caused by removal of CO_2 from the incubation mixture during the continuous argon flow; the content of [^{14}C]bicarbonate was fairly constant in a similar experiment. After 180 min the RuBP oxygenase activity was reduced to only 5% of the original activity and the RuBP carboxylase was diminished to 15%.

The change of the atmosphere to pure oxygen caused a rapid increase of the activity after a significant lag phase; the reactivation of the enzyme molecules did not occur before 10 min. After 60 min of exposure to oxygen the RuBP carboxylase and oxygenase again reached 90–100% of the original activity, indicating the reversibility of this observed oxygen effect. A second cycle of gas changes yielded similar results.

The definite lag phases of the deactivation and the reactivation process were also observed parallel to conformational changes of the enzyme molecules. Preliminary studies of the intrinsic protein fluorescence and studies with the use of the fluorescence indicator 1-anilino-8-naphthalinsulfonate indicated reversible changes.

In the following experiments the conditions under which the enzyme molecules could be protected against the anaerobic inactivation were studied. The results are summarized in table 1. The enzyme is in the fully active state only in the presence of Mg^{2+} and CO_2 [3–7]; the addition of the substrate RuBP causes enzyme inactivation [8], which can be reversed

Table 1
Deactivation and reactivation of RuBP carboxylase-oxygenase during air–argon–oxygen transitions

Enzyme incubation	Enzyme activities in %			
	RuBP case	RuBP oase	RuBP case	RuBP oase
Additions	180 min argon		60 min oxygen	
No addition	100	100	100	100
10 mM $MgCl_2$	15	7	91	100
10 mM $NaHCO_3$	100	100	100	100
10 mM $MgCl_2$ + 10 mM $NaHCO_3$	15	5	93	88
10 mM $MgCl_2$ + 10 mM $NaHCO_3$ + 2 mM β-mercaptoethanol	73	84	79	80
1 mM RuBP	100	100	100	100
1 mM RuBP + 10 mM $MgCl_2$	100	100	100	100

The enzyme was dialysed against 50 mM Tris–sulfate (pH 8.0); the specific activity after preactivation with 10 mM $MgCl_2$ and 10 mM $NaHCO_3$ was 1.15 U/mg protein for RuBP carboxylase (RuBP case) and 0.13 U/mg protein for RuBP oxygenase (RuBP oase), equivalent to 100%

by the addition of an excess of bicarbonate. Therefore, it was interesting to investigate whether a non-activated enzyme, or even a RuBP-treated enzyme sample, would show a similar response to the loss of oxygen.

An enzyme fraction with no additions of $MgCl_2$ or $NaHCO_3$ was gassed with argon or nitrogen for 3 h then preactivated with 10 mM $MgCl_2$ and 10 mM $NaHCO_3$. No loss of RuBP carboxylase and oxygenase activity was observed.

Similar results were obtained with an enzyme fraction which contained 10 mM $NaHCO_3$ or 1 mM RuBP. Neither the RuBP carboxylase nor the RuBP oxygenase activities were lost during the exposure to argon, if the enzyme samples were preactivated with $MgCl_2$ and $NaHCO_3$ prior to the assay.

The question arose, whether only Mg^{2+}–CO_2-activated enzyme would be deactivated by removal of oxygen, or whether the presence of Mg^{2+} or even Mg^{2+} and RuBP would give a similar effect. For both experiments special precautions had to be taken to avoid contamination by CO_2 from air. All solutions were freed of CO_2 and stored with sodium asbestos.

The addition of $MgCl_2$ alone caused the loss of both enzyme activities during anaerobiosis. However, in presence of $MgCl_2$ and RuBP no loss of RuBP carboxylase and oxygenase activity was observed. The procedure for both experiments was the same as above; the enzyme sample was gassed with argon for 180 min then oxygenated for 60 min. Before and after gas changes samples were taken and preactivated with $MgCl_2$ and $NaHCO_3$ and subsequently assayed for both activities. The conclusions to be drawn from these data are:

(i) Mg^{2+} is important for the deactivation process due to oxygen removal;
(ii) The binding of RuBP to the enzyme molecules prevents the Mg^{2+}-dependent deactivation process.

The structural role of the cysteine residues of the enzyme molecules was studied in [9]; modifications of these residues were linked to a decrease of the enzyme activities. Furthermore, the enzyme should be protected against inactivation during the isolation procedure by the presence of sulfhydryl group reducing reagents. Therefore, it was of interest to study whether the presence of β-mercaptoethanol would have a protective effect on the enzyme activities during the air–argon–oxygen transition experiments. Prior to the treatment with argon, 2 mM β-mercaptoethanol was added to an enzyme fraction with 10 mM $MgCl_2$ and 10 mM $NaHCO_3$. After 180 min the activities of the RuBP carboxylase and the RuBP oxygenase were assayed (table 1). RuBP carboxylase was still active (73% of the original activity) and the oxygenase activity also remained very high (84%).

4. Discussion

RuBP carboxylase-oxygenase is activated by Mg^{2+} and CO_2 [3–7]. Recently it was shown that CO_2 may also bind to an effector binding site, which is different to the catalytic site(s) of the enzyme molecules [10]. The results of this paper suggest that the enzyme activity is also controlled by the presence of oxygen; the removal of oxygen is related to the loss of both activities.

The exact role of oxygen in this deactivation and reactivation process is unknown. There are at least two possibilities which should be considered:

(i) Oxygen could bind to a specific binding site of the enzyme;
(ii) Autooxidizable groups of the enzyme, preferentially the sulfhydryl groups of cysteine, could be oxidized.

The experiment with the preactivated enzyme (plus Mg^{2+} and CO_2) revealed that the changes of the enzyme activities occurred relatively slowly. The decline of the enzyme activities was first observed 20 min after the complete removal of 'free' oxygen. The lag phase of this effect could be explained by the assumption that oxygen binds to the enzyme molecules and is lost very slowly. The loss of oxygen is related to a conformational change of the enzyme molecules. In this anaerobic form the enzyme has lost almost all its activities. On the other hand, the addition of oxygen may again trigger a conformational change which allows the enzyme molecules to react with oxygen, thus regaining full activities.

These air–argon–oxygen transition experiments may be useful to study the interaction of the enzyme molecules with oxygen. The possibility exists that oxygen, like CO_2, is involved in two ways: as an effector and as a substrate. The data presented here support clearly that RuBP carboxylase and oxygenase

are affected similarly during the transition changes. Therefore, the speculation that two enzyme activities do not share a common molecule [11] would seem improbable.

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