THE OXYGENASE ACTIVITY OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE FROM RHODOSPIRILLUM RUBRUM[1]

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SUMMARY: Catalysis by pure ribulose bisphosphate carboxylase from Rhodospirillum rubrum, which is a dimer (MW:114,000) lacking small subunits, is inhibited by oxygen. Oxygen is a competitive inhibitor with respect to carbon dioxide. In the absence of carbon dioxide, the enzyme catalyzes the oxygenolytic cleavage of ribulose-1,5-bisphosphate with consumption of one mole of oxygen per mole of 3-phosphoglycerate produced.

Rhodospirillum rubrum is a photo-organotrophic bacterium which photosynthesizes anaerobically. R. rubrum can be cultured heterotrophically in the light on a variety of organic substrates or on hydrogen with carbon dioxide as the major source of carbon (1). The anaerobic photosynthesis as well as simplicity of this prokaryotic organism and its capacity to develop organotrophically or lithotrophically suggest that R. rubrum may be an archetype of autotrophic species (2).

Our laboratory continues to be interested in the evolution of autotrophic species all of which assimilate carbon dioxide through a primary reaction catalyzed by ribulose-1,5-bisphosphate carboxylase [3-phospho-D-glycerate carboxy-lyase (dimerizing) EC 4.1.1.39]. This enzyme is known to occur in three size categories (3): small (MW $\cong$ 120,000), intermediate (MW $\cong$ 360,000) and large (MW $\cong$ 500,000). Interest in the early evolution of autotrophic species has deepened recently with the recognition that enzymes of small and intermediate

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size may be composed of a single type of polypeptide chain (ca. 55,000 daltons). For example, ribulose bisphosphate carboxylases from R. rubrum and Chlorobium limicola forma specialis thiosulfatophilum are dimers and hexamers of 114,000 (4) and 360,000 daltons (5), respectively. In contrast, large enzymes are composed of polypeptide chains (ca. 55,000 daltons) that are possibly homologous to those of small and intermediate enzymes but additionally contain small divergent polypeptide chains (ca. 15,000 daltons) of unknown function (for a review see 6).

I now report the occurrence of an oxygenase activity (7,8) in homogeneous ribulose bisphosphate carboxylase from R. rubrum, an organism which contains high levels of the carboxylase activity when grown anaerobically on CO_2 or butyrate in the light (9,10).

MATERIALS AND METHODS

Ribulose bisphosphate carboxylase was purified to homogeneity from butyrate-grown R. rubrum standard and assayed in the presence of 1 mM EDTA and 6 mM GSH as previously described (10). It was stored at -10° after lyophilization in 0.05 M potassium phosphate, pH 7.4. The specific activity of a solution of lyophilized enzyme was 87% of that for enzyme stored in the frozen state (10). Carboxylase assays were conducted in 10 x 75 mm glass tubes. These were either open to the air or, when alternative gas phases were used, were closed with rubber Suba seals, evacuated, and refilled with the appropriate gas phase via a syringe needle. Reactions were initiated by the addition of tetrasodium ribulose-1,5-bisphosphate (from Sigma Chemical Co.) through a syringe needle.

Manometric probes for oxygenase activity were conducted in Warburg assemblies with each flask containing a wick with 0.1 ml of 10% KOH in the center well to deplete the air atmosphere of carbon dioxide. The main compartment contained: lyophilized R. rubrum enzyme (0.05 mg) which had been dissolved in 0.2 M Ammediol buffer, pH 9.3 (0.4 ml); 10 mM tetrasodium EDTA (0.1 ml); 100 mM $MgCl_2$ (0.1 ml); and 60 mM GSH, pH 9.3 (0.1 ml). After sufficient time to permit temperature equilibration and removal of traces of CO_2 from the air atomsphere,

0.3 ml of 10 mM ribulose-1,5-bisphosphate (or water in the case of controls) was added with rinsing from a side arm. Oxygen consumption was then measured manometrically at 30°. At the conclusion of each run an aliquot of 0.1 ml was rapidly removed from each flask and quickly frozen at -77°. The 3-phosphoglycerate content of each thawed aliquot was subsequently measured spectrophotometrically in the presence of phosphoglycerate kinase, triose phosphate dehydrogenase-isomerase, NADH, ATP (11) and glycerol phosphate dehydrogenase (all from Sigma Chemical Co.).

RESULTS

Oxygen was an inhibitor of ribulose bisphosphate-dependent CO_2 fixation. As evident from Figure 1, it was competitive with respect to CO_2.

Oxygen consumption from an atmosphere of 80% N_2 - 20% O_2 occurred and was ribulose bisphosphate-dependent (Figure 2) and enzyme-dependent (not shown). In the course of these experiments, 1.19 micromoles of ribulose bisphosphate-dependent O_2 consumption was observed in 235 minutes. In accord with the expected stoichiometry for oxygenolytic cleavage of ribulose bisphosphate (7,8), 1.26 micromole of 3-phosphoglycerate was produced and no 3-phosphoglycerate was detectable in flasks not containing ribulose bisphosphate.

DISCUSSION

In 1971 Ogren and Bowes established that O_2 was a competitive inhibitor with respect to CO_2 for soybean ribulose bisphosphate carboxylase (12), leading to the recognition that this enzyme actually catalyzed the cleavage of ribulose bisphosphate at higher partial pressures of oxygen. This cleavage to phospho glycolate and 3-phosphoglycerate was rigorously demonstrated using highly purified spinach ribulose bisphosphate carboxylase (7,8). Quite recently this property has also been found in pure ribulose bisphosphate carboxylase of Euglena gracilis (13). Termed ribulose-1,5-bisphosphate oxygenase, this activity of the carboxylase was presumed to be responsible for phosphoglycolate production during photorespiration (7), a process which consumes oxygen and releases CO_2 thereby decreasing net CO_2 fixation by photosynthesis.

The presence of an oxygenase acitivty in ribulose bisphosphate carboxylase

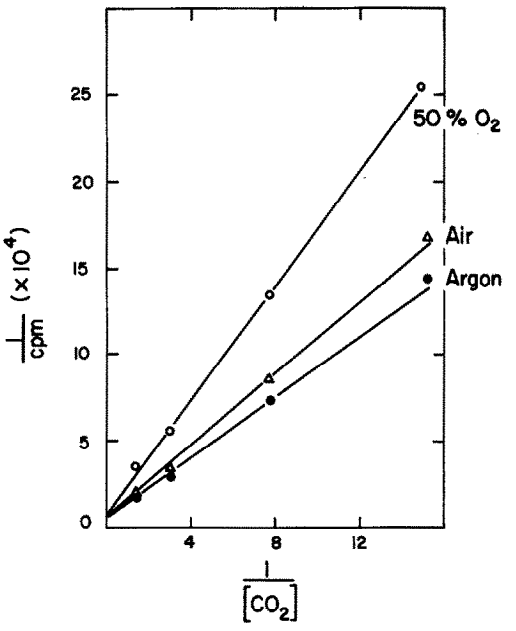

Fig. 1. Reciprocal of acid-stable radioactivity fixed (cpm) x 10^4 versus reciprocal of dissolved [CO_2] in mM. Fixation time was 5 minutes at 30°.

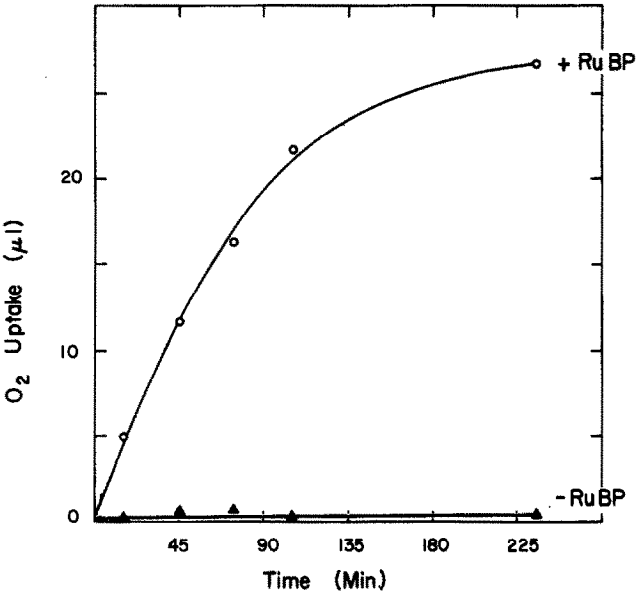

Fig. 2. O_2 consumption in µl at 30° versus time (minutes) in the presence and absence of ribulose bisphosphate (+ RuBP and -RuBP, respectively).

isolated from anaerobically-grown R. rubrum and Chromatium D (14) therefore assumes special significance because these organisms ordinarily encounter little oxygen during photosynthetic growth. Moreover, R. rubrum, at least, presumably became established in evolution before the appearance of oxygen in the atmosphere (10). Perhaps oxygenolytic cleavage of ribulose bisphosphate will indeed prove to be a catalytic property of all ribulose bisphosphate carboxylases because of the susceptibility of the enzyme-bound enolate anion to attack by oxygen (15).

The present work provides the first unequivocal evidence that the presence of small subunits (MW $\simeq$ 15,000) in ribulose bisphosphate carboxylase is not required for oxygenase activity. This enzyme from R. rubrum consists of two closely similar or identical 56,000-dalton subunits but definitely lacks small subunits (4) characteristic of the large enzymes (for a review see 6) including that from Chromatium D (16). The present results thus perpetuate the question of the function of the small subunit found in most ribulose bisphosphate carboxylases.

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