CHEMICAL AND PHYSICAL CHARACTERIZATION OF THE ACTIVATION OF RIBULOSEBISPHOSPHATE CARBOXYLASE/OXYGENASE

MARK I. DONNELLY, V. RAMAKRISHNAN, AND FRED C. HARTMAN

INTRODUCTION

Ribulosebisphosphate carboxylase/oxygenase requires CO₂ and Mg²⁺ for activation; CO₂ reacts with a protein ε-amino group to form a carbamate which is stabilized by binding of Mg²⁺. In the case of the hexadecameric enzyme from spinach, the site of carbamate formation is Lys-201 (Lorimer, 1981). The enzyme from Alcaligenes eutrophus, also hexadecameric, undergoes a substantial change in conformation upon activation as reflected by a decrease in s₂₀,₅ from 17.5 S to 14.3 S (Bowien, Gottschalk, 1982). To assess the generality of these aspects of activation we have characterized the site of carbamate formation in the carboxylase from Rhodospirillum rubrum, a dimeric enzyme, and have examined the shapes of the activated and deactivated forms of the spinach and R. rubrum enzymes in solution by small angle neutron scattering.

METHODS

The quaternary complex of R. rubrum carboxylase-¹⁴CO₂·Mg²⁺·2-carboxy-arabinitolbisphosphate was purified and the activator CO₂ trapped by methylation with diazomethane. After treatment with base to cleave esterified carboxyl groups followed by chymotryptic digestion, two radioactive peptides were purified by standard chromatographic procedures. The peptides' amino acid compositions and sequences were determined by published procedures (Herndon et al., 1982). Shapes of the carboxylases in solution were estimated by comparison of neutron scattering data to that predicted for model structures (Kratky, 1963). The radius of gyration, Rg, and the value of I(0) determined from Guinier plots were used as input data. Activated and deactivated enzymes in H₂O and D₂O were analyzed at varied concentrations, temperatures, and sample-detector distances by use of the 30 meter small angle neutron scattering instrument of the National Center for Small Angle Research at the Oak Ridge National Laboratory. Sedimentation analyses were performed in double sector cells at 9.6°C and 20°C using a Beckman Model E ultracentrifuge equipped with UV scanning optics.

RESULTS AND DISCUSSION

Amino acid analysis of the diazomethane treated complex revealed extensive modification of lysyl residues as well as of cysteine and the acidic residues. Solubilization of the modified complex was achieved by saponification of labile esters followed by digestion with chymotrypsin. The sequences of the two purified peptides were shown to be identical and were highly homologous to that reported for the activation site of the spinach enzyme. (The underlined residues, which were radiolabeled, were identified as Nε-methoxycarbonylylysine):
DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.
DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.
Spinach Peptide (Lorimer, 1981)

Gly-Gly-Leu-Asp-Phe-Thr-Lys-Asp-Asp-Glu-Asn-Val-Asn-Ser-Gln-Pro-Phe

*R. rubrum* Peptide (present study)

Leu-Gly-Gly-Asp-Phe-Ile-Lys-Asp-Glu-Pro-Gln-Gly-Asn-Gln-Pro-Pro-Phe

As aligned, 9 of the 17 residues match. This degree of homology is considerably higher than the overall homology of 28% based on amino acid sequencing of approximately 75% of the total protein (Hartman et al., 1982). Such a localized conservation of sequence in enzymes that differ dramatically in quaternary structure and are obtained from organisms that are evolutionarily very distinct argues strongly for a single mechanism of activation for all species of ribulosebisphosphate carboxylase. However, this proposal must be qualified by consideration of the possible role of the small subunit. Most data regarding this subunit are consistent with it serving to maintain the large subunit in a conformation that can be fully activated (Mziorko, Lorimer, 1983). Recent reconstitution experiments with the enzyme from *Syneccococcus* suggest that the small subunit is absolutely required for the catalytic competence of the large (Andrews, Ballment, 1983). If generally true, the activation of the *R. rubrum* enzyme in the absence of small subunits must involve a mechanism that differs at least in part from that of the complex forms of the enzyme.

Activation of the carboxylase from *A. eutrophus* appears to involve a large conformational change as inferred from the change in $s_{20,w}$ from 17.5 S to 14.3 S (Bowien, Gottschalk, 1982). Our analyses of the solution structures of the enzymes from spinach and *R. rubrum* failed to detect any such large change in conformation upon activation. Sedimentation analysis of the spinach enzyme, employing different preparations of the enzyme and performed at different temperatures, gave $s_{20,w}$ values of 17.8 S for both forms. Similarly, the $s_{20,w}$ values determined for the *R. rubrum* enzyme, 5.9 S and 5.8 S for the activated and deactivated forms, respectively, were not significantly different.

Neutron scattering data substantiated the lack of a large conformational change upon activation of these two enzymes. Scattering by the spinach enzyme in H2O most closely resembled that predicted for a hollow sphere with a radius of gyration of 43.9 Å for both forms (Fig. 1a). In D2O the enzyme resembled a hollow cylinder of axial ratios of 1.0 and 1.1 for the activated and deactivated forms, respectively. The radius of the cavity of this hollow cylinder appeared to expand upon activation, from 12.5 to 13.3 Å, and the cylinder's half height decreased from 52 to 47 Å (Fig. 1b). The outer radius of the cylinder was unchanged, 46.6 Å. While these differences very likely reflect small changes in conformation, they are not consistent with the apparent dramatic change of the *A. eutrophus* enzyme. Similarly, the shape of the *R. rubrum* enzyme was not altered significantly by activation, neutron scattering data being most consistent with that of a solid prolate ellipsoid or cylinder with a radius of gyration of 27.1 Å for both forms (Fig. 1c).
FIGURE 1. Comparison of model structures of tobacco carboxylase (solid lines, Eisenberg et al., 1978) to models from neutron scattering (dashed lines) of spinach enzyme in H2O (a) or D2O (b) or R. rubrum enzyme in D2O (c). Upper models are top views; lower, side views.

Based on data obtained by X-ray diffraction and electron microscopy, other workers have proposed structures for the enzymes from A. eutrophus (Bowien et al., 1980) and tobacco (Eisenberg et al., 1978). While sharing certain features, these two models differ significantly in the shape of the octomeric core of large subunits, the feature which contributes most of the mass and hence will contribute most to the observed scattering. Scattering from both forms of the spinach enzyme closely resembles that anticipated for structures that closely resemble the model of the tobacco enzyme (Fig. 1). The core of large subunits in the model for the A. eutrophus enzyme (not shown) resembles an oblate cylinder with an axial ratio of less than 0.4, a structure very different from that observed by neutron scattering of the spinach enzyme. The structure of the A. eutrophus enzyme was determined under activating conditions and it is possible that this enzyme assumes a structure distinct from that of the higher plant enzymes when activated. If so, such a conformational change is not an essential component of the activation process.

Scattering from the R. rubrum carboxylase resembles that of a prolate ellipsoid or cylinder (Fig. 1c). The similarity between these structures and the dimeric units of the model of the tobacco enzyme suggests that in addition to the partial conservation of primary structure revealed by amino acid sequencing, elements of the quaternary structure of these two evolutionarily very distant enzymes may also have been conserved.
REFERENCES


Herdon CS, Norton IL and Hartman FC (1982) Reexamination of the binding site for pyridoxal 5'-phosphate in ribulosebisphosphate carboxylase/oxygenase from Rhodospirillum rubrum, Biochemistry 21, 1380-1385.


ACKNOWLEDGMENT

Research sponsored jointly by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corp., by the Science and Education Administration of the U.S. Department of Agriculture under Grant 78-59-2474-0-1-161-1, and by the National Science Foundation under Grants PCM-8207516 and DMR-77-24458. We thank Don E. Olins for assistance with the sedimentation experiments.

Author's address: Dr. F. C. Hartman, Biology Division, Oak Ridge National Laboratory, P. O. Box Y, Oak Ridge, Tennessee 37830, U.S.A.