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Isolation and Sequencing of an Active-Site Peptide from *Rhodospirillum rubrum* Ribulosebisphosphate Carboxylase/Oxygenase after Affinity Labeling with 2-[(Bromoacetyl)amino]pentitol 1,5-Bisphosphate†

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ABSTRACT: 2-[(Bromoacetyl)amino]pentitol 1,5-bisphosphate was reported to be a highly selective affinity label for ribulosebisphosphate carboxylase/oxygenase from *Rhodospirillum rubrum* [Fraij, B., & Hartman, F. C. (1982) *J. Biol. Chem. 257*, 3501–3505]. The enzyme has now been inactivated with a ^{14}C-labeled reagent in order to identify the target residue at the sequence level. Subsequent to inactivation, the enzyme was carboxymethylated with iodoacetate and then digested with trypsin. The only radioactive peptide in the digest was obtained at a high degree of purity by successive chromatography on DEAE-cellulose, SP-Sephadex, and Sephadex G-25. On the basis of amino acid analysis of the purified peptide, the derivatized residue was a methionyl sulfonium salt. Automated Edman degradation confirmed the purity of the labeled peptide and established its sequence as Leu-Gln-Gly-Ala-Ser-Gly-Ile-His-Thr-Gly-Thr-Met-Gly-Phe-Gly-Lys-Met-Glu-Gly-Glu-Ser-Ser-Asp-Arg. Cleavage of this peptide with cyanogen bromide showed that the reagent moiety was covalently attached to the second methionyl residue. Sequence homology with the carboxylase/oxygenase from spinach indicates that the lysyl residue immediately preceding the alkylated methionine corresponds to Lys-334, a residue previously implicated at the active site.

We recently reported the synthesis of BrAcNH-pentitol-P_2[1] (an epimeric mixture of the *ribo-* and *arabino*-pentitol derivatives) and showed that it behaves as an affinity label for ribulose-P_2 carboxylase/oxygenase from *Rhodospirillum rubrum*. On the basis of reversibility of inactivation by thiolysis and on the basis of the presence of carboxymethylhomocysteine in acid hydrolysates of the modified enzyme, we concluded that the reaction product is a sulfonium salt of methionine (Fraij & Hartman, 1982). In this report, we describe isolation of the tryptic peptide that contains the reagent moiety and confirm that a methionyl residue is the site of derivatization. Sequence analyses of the purified peptide reveal a homology with an active-site region of the corresponding spinach carboxylase.

Experimental Procedures

Materials

Bicine, ATP, NADH, glutathione, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate phosphokinase, and glycerophosphate dehydrogenase/triose phosphate isomerase were products of Sigma Chemical Co. TPCK-treated trypsin

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[1] Abbreviations: BrAcNH-pentitol-P_2, 2-[(bromoacetyl)amino]pentitol 1,5-bisphosphate; TPCK, *N*-tosylphenylalanine chloromethyl ketone; ribulose-P_2, D-ribulose 1,5-bisphosphate; carboxyribitol-P_2, 2-carboxyribitol 1,5-bisphosphate; Bicine, *N,N*-bis(2-hydroxyethyl)glycine; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; SP, sulfopropyl.

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was purchased from Millipore. Iodoacetic acid, obtained from Aldrich, was recrystallized before use. Carboxyribitol-P_2 was synthesized and purified by ion-exchange chromatography as described by Pierce et al. (1980). ^{14}C-Labeled BrAcNH-pentitol-P_2 (sp act. 1.6×10^6 cpm/μmol) was obtained by acylation of 2-aminopentitol-P_2 with *N*-hydroxysuccinimide bromo[^{14}C]acetate (Fraij & Hartman, 1982). *R. rubrum* ribulose-P_2 carboxylase/oxygenase, a dimer of 56 000-dalton subunits (Tabita & McFadden, 1974), was purified to homogeneity as reported previously (Schloss et al., 1979).

Methods

Carboxylase Assays. Carboxylase activity was determined at 25 °C by the spectrophotometric method of Racker (1963) with slight modification (Norton et al., 1975). The enzyme's concentration was based on the A_{280nm} given an $E^{1\%}_{1cm}$ of 12.0 (Stringer et al., 1981).

Radioactivity. Radioactivity was measured with a Packard 3255 liquid scintillation spectrometer. Samples were counted in 10 mL of ACS scintillation fluid (Amersham/Searle).

Amino Acid Analyses. Total hydrolysis of peptides was achieved in evacuated (<50 μmHg) sealed tubes with 6 N HCl/0.01 M 2-mercaptoethanol at 110 °C for 21 h. Hydrolysates were dried on a Speed Vac concentrator (Savant Instruments Inc.) and subjected to chromatography on a Beckman 121 M amino acid analyzer using Beckman's "3-hour-single-column system".

Carboxymethylation and Trypsin Digestion. The carboxylase samples that had been treated for 6 h with ^{14}C-labeled reagent under protective and inactivation conditions (see below) were carboxymethylated with iodoacetic acid as described previously (Schloss et al., 1978). Subsequent to dialysis against 0.01 M ammonium bicarbonate, the carboxymethylated enzymes were digested with 1% (w/w) trypsin for 12 h at 40 °C, at which time an additional 1% trypsin was added. Twelve hours later, the samples were frozen and stored at −20 °C until needed.

Sequence Analyses. Peptides were subjected to automated Edman degradation with a Beckman 890C, the vacuum system of which was modified according to Bhown et al. (1980). A liquid nitrogen cold trap was inserted between the low vacuum pump and the vacuum manifold. Polybrene (2 mg) was added to the peptide to reduce its extraction from the reaction cup (Tarr et al., 1978). Sequencing was carried out in a Quadrol buffer system utilizing the program of Bhown et al. (1980) with several changes. The buffer concentration was reduced from 0.5 to 0.1 M, the drying step after delivery of phenyl isothiocyanate was increased from 20 to 60 s, and the coupling steps and drying steps after delivery of benzene/ethyl acetate were from Beckman's peptide program No. 030176. Half of the fraction from each cycle was converted to the phenylthiohydantoin derivative for identification by high-performance liquid chromatography (Laboratory Data Control) and the rest hydrolyzed in base for quantitation as free amino acids on the amino acid analyzer (Smithies et al., 1971). Threonine and arginine appear as α-aminobutyric acid and ornithine, respectively, in base hydrolysates. Aspartic and glutamic acid were distinguished from the corresponding amides by high-performance liquid chromatography of the phenylthiohydantoin derivatives.

Incorporation. Determination of the extent of covalent incorporation by precipitation of the protein on filter paper with trichloroacetic acid was an adaptation of a method described by Bollum (1968). Only protein-bound reagent, because of its acid insolubility, remains associated with the paper disk. The filter paper disks, on which aliquots of the reaction mixtures had been applied and the protein precipitated by placement of the disks in trichloroacetic acid (see below), were washed twice for 10 min with 500-mL portions of 5% trichloroacetic acid at 4 °C, twice with 500-mL portions of ethanol/diethyl ether (1:1 v/v) at 4 °C, and twice with 500-mL portions of diethyl ether at room temperature. Finally, the disks were air-dried and counted in 10 mL of scintillation fluid. The degree of quenching by the paper disk was determined by counting aliquots of the stock reagent both in solution and dried on filter paper (not carried through the washing procedure just described).

Polyacrylamide Gel Electrophoresis. Samples of the enzyme that were taken during the course of the modification, added to glycerol, and stored in liquid nitrogen (see below) were subjected to electrophoresis on 7.5% polyacrylamide gels in Tris-HCl (pH 9.5). An apparatus from Canal Industrial Corp. was used according to their instructions. Gels were stained with Amido Schwaz and destained electrophoretically. Stained protein bands were quantified by scanning the gels at 540 nm with a Gilson Holochrome spectrophotometer. The amount of radioactive label associated with each band was determined by taking appropriate slices of the gel, which were solubilized directly in scintillation vials by incubation with 0.5 mL of 30% hydrogen peroxide for 12 h at 40 °C. The dissolved gel slices were then mixed with 10 mL of scintillation fluid and counted.

Treatment of R. rubrum Ribulose-P_2 Carboxylase/Oxygenase with BrAcNH-pentitol-P_2. A 30-mL solution of enzyme (5 mg/mL, 0.089 mM subunit) in 50 mM Bicine/66 mM $NaHCO_3$/5 mM $MgCl_2$/0.1 mM EDTA (pH 8.0) was divided into 20-, 8-, and 2-mL portions, the latter serving as control. Carboxyribitol-P_2 (2.2 mg of the lithium salt) was added to the 8-mL sample, giving a final protector concentration of 0.7 mM. Reactions were initiated by the additions of 218 and 88 μL of 23 mM ^{14}C-labeled BrAcNH-pentitol-P_2 (free acid in water) to the 20- and 8-mL samples of enzyme, respectively, giving a final reagent concentration of 0.25 mM. Periodically, 5-μL aliquots of the control and reaction mixtures were diluted 10-fold with the Bicine buffer, and 5 μL of these dilutions was promptly assayed for carboxylase activity. Separate aliquots (100 μL) of the reaction mixtures were mixed with 100 μL of glycerol containing 5 μL of 1 M 2-mercaptoethanol, and the samples were quickly frozen and stored in liquid nitrogen prior to their inspection by polyacrylamide gel electrophoresis. Aliquots (25 μL) of the reaction mixtures were also applied to Whatman 3MM circular filter paper (2.3 cm in diameter), which were then immersed immediately in 500 mL of ice-cold 10% aqueous trichloroacetic acid for subsequent incorporation measurements. All of the filter paper samples taken during the progression of the modification were placed in the same vessel of trichloroacetic acid, where they could remain for at least 24 h without variation in the incorporation data subsequently obtained.

After a 6-h reaction time, the unprotected sample retained only 20% of its initial activity, whereas the sample protected with carboxyribitol-P_2 was 85% active. At that time, 6 mL of the protected sample and 16 mL of the unprotected sample were chilled and dialyzed at 4 °C against the Bicine buffer (pH 8.0) containing 10 mM sodium phosphate; these samples of derivatized enzyme were carboxymethylated and digested with trypsin for the peptide characterization experiments.

Results

Inactivation of Ribulose-P_2 Carboxylase/Oxygenase with ^{14}C-Labeled BrAcNH-pentitol-P_2. As described under Experimental Procedures, modification of the enzyme was

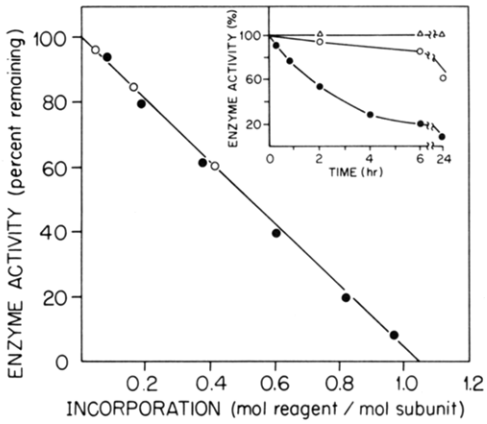

FIGURE 1: Covalent incorporation of BrAcNH-pentitol-P_2 into ribulose-P_2 carboxylase as a function of extent of inactivation. Time courses for the reactions are shown in the inset. Inactivations were carried out in the absence (●) and presence (○) of carboxyribitol-P_2; the control (△) retained full activity. Experimental details are provided in the text.

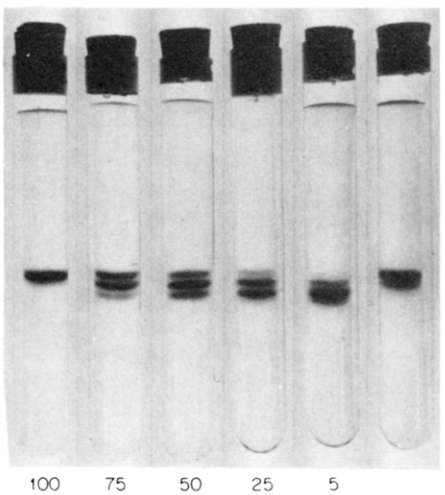

FIGURE 2: Polyacrylamide gel electrophoresis (nondenaturing conditions) of ribulose-P_2 carboxylase inactivated to various extents by BrAcNH-pentitol-P_2. The numbers beneath the gels are the percentages of the initial enzymic activity remaining. The sample on the extreme left is the untreated control, and the sample on the extreme right is the 95%-inactivated enzyme after incubation with 0.1 M 2-mercaptoethanol (pH 8.0) for 8 h, which restores 85% of the lost activity.

monitored by the time course of inactivation (Figure 1, inset), the covalent incorporation of reagent (Figure 1), and analytical gel electrophoresis (Figure 2). The incorporation extrapolated to complete inactivation is 1.1 mol of reagent/mol of catalytic subunit, which indicates a high degree of selectivity for a single residue. In the presence of the competitive inhibitor carboxyribitol-P_2, a good protector, the inactivation that does occur also appears directly proportional to the incorporation. Specificity is also demonstrated by the appearance of the inactivated enzyme as discrete new species when viewed by polyacrylamide gel electrophoresis (Figure 2). The pattern observed in partially inactivated samples is that anticipated for a dimeric enzyme with a functional catalytic site on each subunit. The previously observed reversal of alkylation by thiolysis as based on enzymic activity measurements can also be demonstrated by electrophoresis. The enzyme species with an electrophoretic mobility intermediate between that of the native and fully inactivated enzyme presumably has but one of its two active sites derivatized and thus retains 50% of its original activity. Consistent with this view, the ratio of the three bands in the gel of 50%-inactivated enzyme is 1:2:1

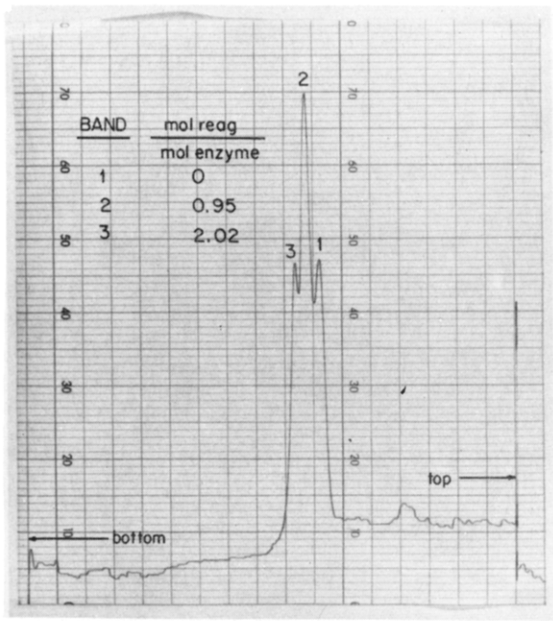

FIGURE 3: Absorbancy scan of the gel shown in Figure 2 in which the sample was 50% inactivated. The quantity of protein represented by each peak was calculated from its area and a knowledge of the total amount of protein applied to the gel; the amount of radioactivity associated with each band of protein was determined by counting appropriate slices (see text). Stoichiometries shown in the figure were thus obtained.

(native:intermediate:fully inactivated) as determined by scanning, and the specific radioactivity of the fully inactivated species is twice that of the intermediate species as determined by slicing the gel and counting the solubilized slices (Figure 3).

Isolation of Active-Site Peptide. A tryptic digest (56 mg; 1 μmol of subunit; 1.3×10^6 cpm) of enzyme that had been 80% inactivated with ^{14}C-labeled BrAcNH-pentitol-P_2 and carboxymethylated as described under Experimental Procedures was fractionated on DEAE-cellulose (Figure 4A). Fractions containing the single radioactive peak (1.0×10^6 cpm, 77%) were pooled, lyophilized to dryness, and dissolved in 6 mL of 0.01 M phosphoric acid/6 M urea (adjusted to pH 3.0 with 1 N NaOH). This material was then chromatographed in succession on SP-Sephadex and Sephadex G-25; the only labeled peptide observed was recovered in 77% and 91% yield, respectively, from these latter two columns (Figure 4B,C). The peptide (7×10^5 cpm, 54% overall recovery) thus obtained appeared about 90% homogeneous as judged by sequence analysis (see below).

The tryptic digest of enzyme that had been treated with the reagent in the presence of carboxyribitol-P_2 and had retained 85% of its initial activity was also inspected by chromatography on DEAE-cellulose. The only labeled peptide observed coeluted with the one present in the digest of inactivated carboxylase (data not shown).

Characterization of Active-Site Peptide. The amino acid composition of the purified peptide is shown in Table I. On the basis of the total amount of the peptide (434 nmol) as determined by amino acid analysis, its specific activity (1.6×10^6 cpm/μmol) agrees precisely with that of the modifying reagent; thus the peptide contains only one derivatized amino acid. The appearance of carboxymethylhomocysteine, homoserine, and homoserine lactone in the acid hydrolysate of the peptide (Table I) supports our earlier conclusion that the modified residue is a methionylsulfonium salt (Fraij & Hartman, 1982).

Table I: Amino Acid Composition of Active-Site Peptide[a]

amino acid	nanomoles found	number of residues (nearest integer)
Cys(Cm)[b]	none	
Asp	1.13[c]	1.0[d] (1)
Thr	2.06	1.8 (2)
Ser	3.30	2.9 (3)
Hse	0.17	0.15
Glu	3.21	2.8 (3)
Pro	none	
Hcy(Cm)	0.24	0.21
Gly	6.44	5.7 (6)
Ala	1.05	0.93 (1)
Val	trace	
Met	1.27	1.1 (1)
Ile	0.99	0.88 (1)
Leu	1.10	0.98 (1)
Tyr	trace	
Phe	1.21	1.1 (1)
His	0.96	0.85 (1)
Hse lactone	0.14	0.12
Lys	1.22	1.1 (1)
Trp	none	
Arg	1.15	1.0 (1)

[a] Aliquot subjected to analysis contained 1813 cpm (0.26%) of the total 7×10^5 cpm in the isolated peptide. [b] Abbreviations: Cys(Cm), carboxymethylcysteine; Hse, homoserine; Hcy(Cm), carboxymethylhomocysteine. [c] The finding of 1.13 nmol in 0.26% of sample obtained (see footnote *a*) reflects 434 nmol of peptide with a specific activity of 1.6×10^6 cpm/μmol. [d] Arbitrarily set as 1.0.

Data from automated Edman degradation of the labeled peptide establish its sequence as follows (Table II):

Leu-Gln-Gly-Ala-Ser-Gly-Ile-His-Thr-Gly-Thr-Met-Gly-Phe-Gly-Lys-
Met-Glu-Gly-Glu-Ser-Ser-Asp-Arg

Apparently, the sulfonium salt reverts to methionine during the sequencing, since methionine was observed at cycles 12 and 17. The resistance of the peptide bond between Lys-16 and Met-17 to trypsin suggests that the anionic reagent moiety is bonded to Met-17. Trypsin-catalyzed hydrolysis is known to be retarded by acidic residues adjacent to the lysyl or arginyl residue under attack (Smyth, 1967).

For direct determination of which methionyl was labeled, the peptide was cleaved with cyanogen bromide, to which methionyl sulfonium salts are resistant (Link & Stark, 1968). The resulting mixture was then analyzed by automated Edman degradation (Table III). At cycle 1, Leu and Gly were released; at cycle 2, Gln and Phe were released; and at cycle 3, only Gly was released. Thus, cleavage occurred at Met-12, establishing Met-17 as the alkylated residue.

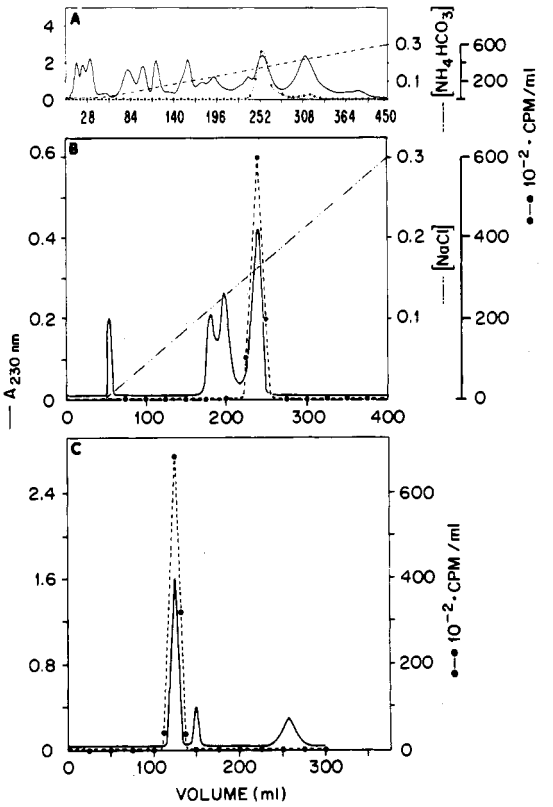

FIGURE 4: Purification of radioactive peptide from a tryptic digest of ribulose-P_2 carboxylase after inactivation with ^{14}C-labeled BrAcNH-pentitol-P_2. (A) Anion-exchange chromatography of the digest (56 mg) on a Whatman DE-52 column (1 cm × 25 cm) equilibrated with 0.01 M NH_4HCO_3 (pH 8.1) and eluted with 50 mL of 0.01 M NH_4HCO_3 followed by a 400-mL linear gradient of 0.01–0.3 M NH_4HCO_3 (pH 8.1). (B) Cation-exchange chromatography of the radioactive peak from the DE-52 column on a SP-Sephadex column (1 cm × 25 cm) equilibrated with 0.01 M sodium phosphate (pH 3.0) and eluted with a 400-mL linear gradient of 0–0.3 M NaCl in the phosphate buffer. (C) Gel filtration of the radioactive peak from the SP-Sephadex column on a Sephadex G-25 column (1.7 cm × 220 cm) in 0.01 M NH_4HCO_3. Additional experimental details are provided in the text.

Discussion

Prior to the design of BrAcNH-pentitol-P_2, the only thoroughly documented example of affinity labeling of *R. rubrum* ribulose-P_2 carboxylase/oxygenase was with pyridoxal phosphate (Whitman & Tabita, 1978a,b). On the basis of sequence

Table II: Sequence Analysis of Labeled Peptide (50 nmol)[a]

amino acid	cycle no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Asp																		0.4	0.4				*1.1*	
Thr									*11.6*	2.5	*10.1*	1.9	1.0	0.6										
Ser	1.1			0.7	*5.2*	2.1															*1.6*	*1.9*	1.3	0.3
Glu						0.6									0.3			*3.6*	0.2	*3.8*	1.9	0.9	0.4	0.3
Gln		*12.4*	2.6	0.8															0.9					
Gly	2.6	1.7	*24.8*	4.7	2.0	*20.5*	4.0	1.4	1.1	*12.9*	4.5	2.3	*10.3*	4.1	*9.7*	3.4	1.6	1.6	*4.2*	3.2	1.3	0.2	0.3	0.3
Ala				*24.4*	3.8	0.9										0.7								
Met											0.6	*15.1*	3.1	1.1	0.7		*6.1*	2.7	0.8	0.3				
Ile							*19.4*	2.9																
Leu	*28.5*																							
Phe														*10.4*				0.2						
His								*3.7*	0.7	0.2														
Lys															0.2	*4.0*	1.4	0.7	0.7					
Arg																								*0.6*

[a] Numbers in italics denote major residue released at each cycle.

Chart I

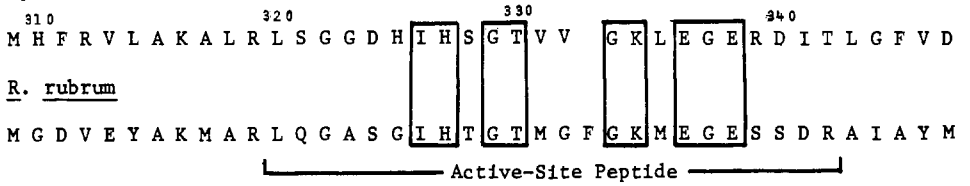

Table III: Sequence Analysis of Labeled Peptide after CNBr Cleavage (10 nmol)[a]

amino acid	cycle no. 1	2	3
Gln		*1.3*	0.6
Gly	*4.1*	0.6	*6.9*
Leu	*4.8*	0.1	
Phe		*3.2*	0.8

[a] Numbers in italics denote major residues released at each cycle.

homology, the lysyl residue preferentially labeled by pyridoxal phosphate corresponds to Lys-175 of the spinach enzyme (Herndon et al., 1982). This residue had been implicated at the active site with three different affinity labels (Hartman et al., 1978; Spellman et al., 1979), and given the observed species invariance and conservation of sequence, Lys-175 seems a likely candidate for catalytic functionality (Herndon et al., 1982). The present characterization study was prompted by the finding that BrAcNH-pentitol-P_2 reacts with a residue other than the one targeted by pyridoxal phosphate and thus should permit localization of another region of the polypeptide chain that encompasses a portion of the active site.

The labeling pattern observed (Figure 2) by gel electrophoresis of enzyme partially inactivated by BrAcNH-pentitol-P_2 is consistent with the established dimeric structure of *R. rubrum* carboxylase (Tabita & McFadden, 1974; Schloss et al., 1979) in which each subunit contains a catalytic site (Herndon et al., 1982). The virtually absolute specificity of the reagent for a single site as revealed by ion-exchange chromatography of the total tryptic digest (Figure 4A) assures that the purified peptide contains the residue whose modification correlates with loss of enzymic activity. On the basis of amino acid analysis and cyanogen bromide cleavage of the labeled peptide, the derivatized amino acid is clearly a sulfonium salt of methionine.

On the basis of visual inspection, the active-site peptide from *R. rubrum* carboxylase corresponds to the sequence from residue 320 to residue 342 in the spinach carboxylase (see Chart I). The total sequences of the highly homologous (~90%) carboxylases from spinach and maize were deduced from characterization of their respective genes (McIntosh et al., 1980; Zurawski et al., 1981), and the above sequence of the *R. rubrum* enzyme adjacent to the active-site peptide was determined directly on purified cyanogen bromide fragments (Hartman et al., 1983). In contrast to the extreme conservation of primary structure among the plant carboxylases, the total homology between the *R. rubrum* and spinach carboxylase is only about 28%. Thus, stretches of sequences that are similar are likely to be functionally significant.

As seen in the sequences shown above, the methionyl residue subject to affinity labeling by BrAcNH-pentitol-P_2 corresponds to Leu-335 in the spinach enzyme. Of the 13 residues from position 326 through 338, nine are identical (enclosed in boxes) in the two species. Given the species variance, Met-335 cannot be functional in catalysis, but it may be structurally important. In terms of hydrophobicity, methionine and leucine are quantitatively quite similar (Nozaki & Tanford, 1971) and are frequently interchanged at specific loci among homologous proteins [see discussions by Smith (1970) and Torchinsky (1981)]. Consistent with a structural role of Met-335 in the *R. rubrum* carboxylase are recent results obtained with the affinity label 2-(*N*-chloroamino)-2-deoxypentitol 1,5-bisphosphate, which differs from the reagent used in the present study only in a chlorine substituent in place of the bromoacetyl group (Christeller & Hartman, 1982). This reagent inactivates the *R. rubrum* enzyme as a consequence of the selective oxidation of a methionyl residue to the corresponding sulfoxide; the inactivation primarily reflects a reduced V_{max} with little change in K_m for ribulose-P_2. Thus, conversion of a neutral, hydrophobic thioether to the strongly dipolar sulfoxide (a rather small steric change) substantially lowers the catalytic efficiency. The assumption is made that both the chloro and bromoacetyl compounds react with the same methionine; this appears reasonable on the basis of the mechanisms of their reactions with methionine and on the basis of considerations of molecular models, as well as the experimental observation that the spinach enzyme (which contains leucine rather than methionine at position 335) is less sensitive to oxidation by the chloroamine.

Perhaps our most significant new finding is the interspecies conservation of Lys-334, the residue immediately preceding the alkylated methionine. In the spinach enzyme, Lys-334 (Zurawski et al., 1981) was thought to occupy a position within the active-site region on the basis of preferential alkylation by the affinity label 3-bromo-1,4-dihydroxy-2-butanone 1,4-bisphosphate (Hartman et al., 1978). Although this reagent also inactivated the carboxylase from *R. rubrum* via lysyl modification (Schloss & Hartman, 1977), lack of specificity as judged by the inspection of tryptic digests (F. C. Hartman, unpublished data) precluded ascertaining if the same lysyl residue was involved. With the present observation that Lys-334 is conserved among species as evolutionarily diverse as spinach and *R. rubrum*, the postulate that this lysine is an active-site component is greatly strengthened, and furthermore, the likelihood of catalytic involvement arises.

The stringent specificity of BrAcNH-pentitol-P_2 for Met-335 of *R. rubrum* carboxylase and its failure to react with the adjacent lysyl side chain suggest that within the reagent–enzyme complex there is rather precise and constrained alignment between the bromoacetyl group and the attacking thioether. Consistent with a lack of mobility of the reagent within the substrate binding site is the inertness of the spinach enzyme to BrAcNH-pentitol-P_2 despite the presumed close proximity of the ϵ-amino group of Lys-334; leucine, of course, is not subject to alkylation. Under conditions where *R. rubrum* ribulose-P_2 carboxylase is completely inactivated by BrAcNH-pentitol-P_2, the spinach enzyme retains full activity (data not shown). In view of the documented differences in structural and functional properties of the plant and *R. rubrum* carboxylases (McFadden & Tabita, 1974; Jordan & Ogren, 1981; Christeller, 1981; Hartman et al., 1983), some variability in their active-site chemistry is not unexpected. 3-Bromo-1,4-dihydroxy-2-butanone 1,4-bisphosphate and BrAcNH-

pentitol-P_2 complement each other by revealing a region of homology, which encompasses a portion of the active site, that could not be detected with either reagent alone.

Registry No. BrAcNH-pentitol-P_2, 84520-93-4; ribulosebisphosphate carboxylase, 9027-23-0; methionine, 63-68-3.

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