Biochimica et Biophysica Acta, 662 (1981) 181–189
Elsevier/North-Holland Biomedical Press

BBA 69410

ESSENTIAL HISTIDINE RESIDUES OF RIBULOSEBISPHOSPHATE CARBOXYLASE INDICATED BY REACTION WITH DIETHYLPYROCARBONATE AND ROSE BENGAL

A.S. BHAGWAT and J. RAMAKRISHNA *

Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400 085 (India)

(Received March 24th, 1981)
(Revised manuscript received July 7th, 1981)

Key words: Ribulosebisphosphate carboxylase; Histidine residue; Diethylpyrocarbonate; Rose bengal

Modification of ribulosebisphosphate carboxylase (3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39) by diethylpyrocarbonate or rose bengal-sensitized photooxidation caused rapid inactivation of the enzyme. The photooxidation proceeded following pseudo-first-order reaction kinetics showing a maximal value at pH 8.0. The fully activated enzyme was more sensitive to photooxidation as compared to the unactivated enzyme. The enzyme partially inactivated by photooxidation was fully sensitive to the positive effectors. The photooxidised enzyme showed a characteristic increase in absorbance at 250 nm which was dependent on the extent of inactivation. The kinetic analyses and correlation of the spectral changes with the activity indicated that the inactivation by diethylpyrocarbonate resulted from the modification of an average one histidine residue/70 000-dalton combination of large and small subunit. Sulfhydryl, lysine and tyrosine residues were not modified by diethylpyrocarbonate treatment. Ribulosebisphosphate and some effectors of the enzyme offered significant protection against diethylpyrocarbonate modification indicating that diethylpyrocarbonate was interacting with the enzyme at or near the active site.

Introduction

Carboxylation of ribulose 1,5-bisphosphate is the first step in the photosynthetic carbon reduction cycle [1]. The important studies that paved the way for definitive enzymatic studies were earlier reported by Calvin's and Horecker's groups [2,3]. Ribulosebisphosphate carboxylase was shown to possess ribulosebisphosphate oxygenase activity [4]. The ribulosebisphosphate/oxygenase (3-phospho-D-glycerate carboxy-lyase (dimerizing), EC 4.1.1.39) appears to catalyse carboxylation and oxygenation reactions by homologous mechanism and a common binding site exists for the acceptor substrate ribulosebisphosphate. The carboxylase enzyme from spinach has a molecular weight of 557 000 and is composed of non-identical subunits [5]. A 70 000-dalton combination of large and small subunit is considered as a active or a protomeric unit [5].

Even though the basic mechanisms for carboxylation and oxygenation of ribulosebisphosphate have been proposed and some evidence in support of these mechanisms has been obtained, the details of reaction mechanism and active site chemistry of the enzyme are not completely understood [6,7]. The earlier reports [8–11] have suggested that lysine and sulfhydryl residues are essential for activity of the carboxylase enzyme. The presence of arginine at the active site of bacterial [12] and plant enzyme [13] has been demonstrated.

Using two histidine selective reagents, diethylpyrocarbonate and an anionic dye rose bengal, we present evidence to show that histidine residues of

* Deceased.

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid).

ribulosebisphosphate carboxylase are essential for activity.

Materials and Methods

Materials

Rose bengal was obtained from B.D.H. (U.K.). $NaH^{14}CO_3$ was a product of this Research Centre. All other chemicals were purchased from Sigma Chemical Co. Diethylpyrocarbonate was diluted with ethanol to the required concentration which was calculated using an extinction coefficient of $3 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ at 240 nm.

Methods

Enzyme preparation. Ribulosebisphosphate carboxylase from spinach leaves was purified to homogeneity according to the method of Wishnick and Lane [14]. Protein concentration was estimated by multiplying A_{280nm} in a cuvette of 1-cm light path by a factor 0.61 mg/ml [5]. All spectrophotometric measurements were done in an Aminco DW-2a spectrophotometer.

Enzyme activity. The activation of the enzyme and assay of carboxylase and oxygenase reactions were done according to the method of Lorimer et al. [15].

Photooxidation of the enzyme using rose bengal. The enzyme was irradiated in the presence of indicated concentrations of rose bengal using a slide projector (500 W lamp) at a distance of 12 cm. In some cases samples were reactivated with 10 mM HCO_3^- and 20 mM $MgCl_2$ after photooxidation. The detailed conditions for photooxidation are given in the legends of the respective figures.

To study the influence of various positive effectors on the photooxidised enzyme, the irradiations were done at 1 mM HCO_3^- and 10 mM $MgCl_2$. Rose bengal was used at a concentration of 0.01 mg/ml. The enzyme was irradiated until 30% residual activity remained. Control and photooxidised enzyme samples after suitable dilution with 'CO_2-free' buffer were preincubated with 0.1 mM NADPH, 0.1 mM phosphogluconate, and 5 mM P_i in 50 mM Tris-HCl (pH 7.5) for 10 min. The enzyme activity was assayed at 1 mM HCO_3^-. The reactions were initiated by addition of 20 µl enzyme and were terminated after 30 s.

Chemical modification using diethylpyrocarbonate. The fully activated enzyme in either 20 mM Tricine-KOH or Bicine-KOH buffer (pH 8.0) containing 10 mM HCO_3^- and 20 mM $MgCl_2$ was used for the modification studies. The modification of the enzyme with diethylpyrocarbonate was either done in Hepes-KOH, pH 6.1, or Tricine-KOH, pH 7.0.

For studies on protection of the enzyme against diethylpyrocarbonate modification, the enzyme was preincubated with various effectors for 5 min at pH 8.0 and the diethylpyrocarbonate modification was done at pH 7.0 in Tricine-KOH buffer.

The total number of histidine residues modified was calculated from the absorbance at 240 nm ($\Delta\epsilon 240 = 3200$ $cm^{-1} \cdot M^{-1}$) as described by Miles [16].

Reversal of diethylpyrocarbonate inhibition. The enzyme (6 µM) was modified by diethylpyrocarbonate at pH 6.2 in Hepes-KOH buffer/10 mM HCO_3^-/20 mM $MgCl_2$ until 30 or 48% residual activity remained. To one batch of control and diethylpyrocarbonate-treated enzyme was added 0.4 M NH_2OH, pH 7.2 (final concentration)/2 mM dithiothreitol and incubated for 16 h at 4°C. All samples were passed through Sephadex G-25 column to remove NH_2OH and assayed for both carboxylase and oxygenase activity.

DTNB titration of sulfhydryl groups. The titration method was basically that of Ellman [17]. A molar extinction coefficient of 13 600 at 412 nm was used for calculating the number of -SH groups blocked per 557 000-dalton protein.

Spectrophotometric determination of Schiff base. The aldimine formation between pyridoxal phosphate and ribulosebisphosphate carboxylase with and without diethylpyrocarbonate modification was determined according to the method described by Paech et al. [30]. A molar extinction coefficient of 5800 was used for calculations.

Pyridoxal phosphate protection of lysine residues and reversal of pyridoxal phosphate inhibition after diethylpyrocarbonate modification. The fully activated enzyme in 20 mM bicine-KOH (pH 8.0)/10 mM HCO_3^-/10 mM $MgCl_2$ was used for modification of the enzyme by pyridoxal phosphate. The enzyme solution was diluted after pyridoxal phosphate treatment with an equal volume of 100 mM Hepes-KOH (pH 6.1) buffer/10 mM HCO_3^-/20 mM $MgCl_2$. Diethylpyrocarbonate modification and reversal of

pyridoxal phosphate inhibition was done as described in the legend to Table I. Appropriate controls were included to check reversal of pyridoxal phosphate inhibition by NH_2OH.

Results

Photooxidation of the enzyme sensitised by rose bengal. The carboxylase activity was strongly inhibited by rose bengal-sensitized photooxidation of the enzyme (Fig. 1). The inactivation followed pseudo-first-order reaction kinetics. Rose bengal had no effect on dark controls nor was the enzyme affected by light in absence of the dye.

To ascertain the specificity of rose bengal-sensitized photooxidation the following experiments were done.

Effect of pH. The effect of pH on photooxidation of the enzyme is shown in Fig. 2. The pH profile of the inactivation rate by photooxidation is explicable in terms of ionization of a group having a pK_a value about 7.0, possibly histidine. However, reasons for decrease in the rate of photooxidation above pH 8.0 are not clear.

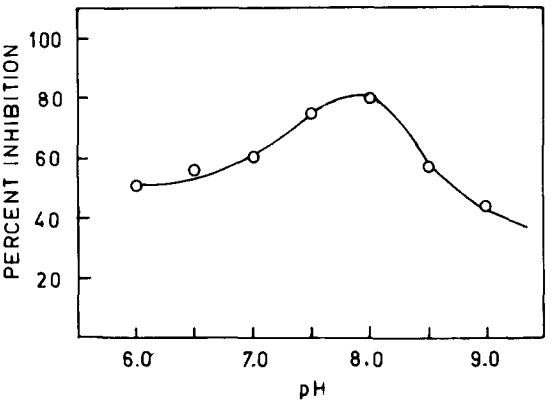

Fig. 2. Effect of pH on rose bengal-sensitized photooxidation of the enzyme. The enzyme in Tes-KOH buffer/10 mM $MgCl_2$/10 mM $NaHCO_3/10^{-5}$ M EDTA was photooxidised using 7.5 μg/ml rose bengal at the indicated pH values. The carboxylase activity was determined after 3 min irradiation of the samples. An identical sample for each pH value kept in dark serves as control.

Effect of activation of the enzyme. The enzyme activated with HCO_3^- and Mg^{2+} was more sensitive to photooxiation as compared to the unactivated enzyme (Fig. 3). Since in the absence of CO_2 and Mg^{2+} the carboxylase is essentially inactive [1], it

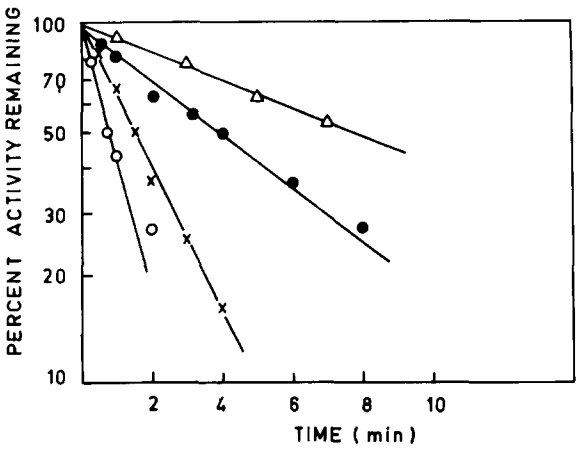

Fig. 1. Time course of inactivation of the enzyme at different rose bengal concentrations. The enzyme (6.1 μM) in 50 mM Tris-HCl, pH 7.8/10 mM $NaHCO_3$/20 mM $MgCl_2/10^{-5}$ M EDTA was irradiated with 2.5 μg/ml (△), 5 μg/ml (•), 10 μg/ml (×) and 20 μg/ml (○) of dye for the indicated time period. An identical sample kept in dark was taken as 100%. The enzyme samples were reduced with 1 mM dithiothreitol after photooxidation.

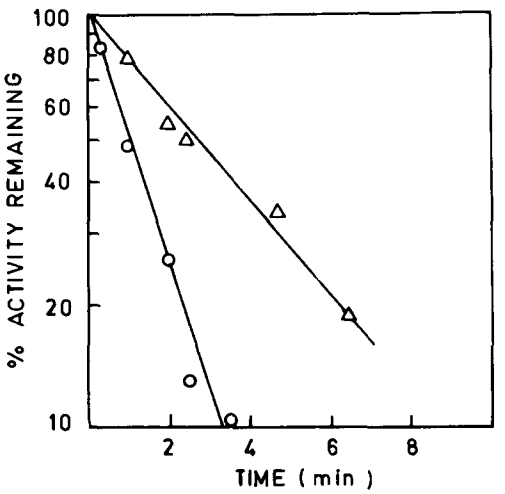

Fig. 3. Effect of activation state of the enzyme on the rate of photooxidation. The enzyme with (○) and without (△) activation by 10 mM $NaHCO_3$/20 mM $MgCl_2$ was photooxidised at 0.01 mg/ml rose bengal. Aliquots withdrawn at the specified time intervals were assayed for the carboxylase activity.

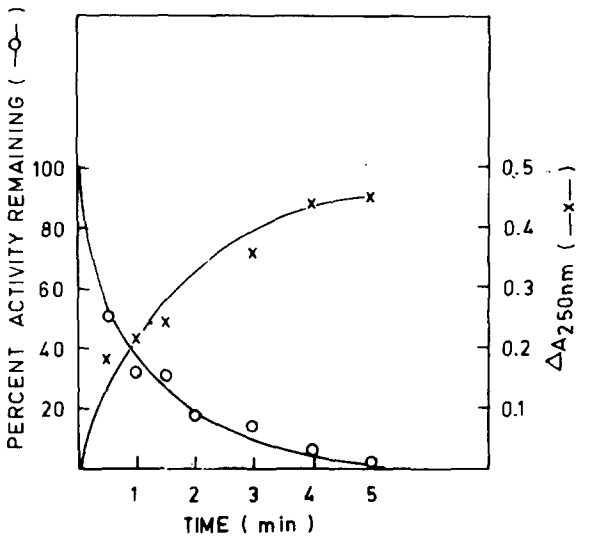

Fig. 4. Correlation of 250 nm increase in absorbance with percent inhibition of the enzyme activity following photooxidation by rose bengal. The enzyme (1.3 mg/ml) was photoxidised at 0.01 mg/ml rose bengal. The aliquots withdrawn at the indicated time intervals were assayed for enzyme activity and 250 nm increase in absorbance after removal of the dye using a Sephadex G-25 column.

seems that the residues involved in photooxidation were more accessible in the active conformation of the enzyme.

Spectral characteristics of the photooxidised enzyme. At 278 nm the changes in the spectral characteristics were not observed, however, at 250 nm the photooxidised enzyme showed a distinct increase in absorbance as compared to the dark control. Increase in absorbance at 250 nm seems to relate to the number of histidine residues photooxidized [18]. The correlation observed in Fig. 4 between the increase in absorbance and the extent of inactivation of the enzyme suggested that the loss of enzyme activity by photooxidation may be due to the modification of the critical histidine residues.

Substrate protection and influence of effectors on photooxidised enzyme. At 1 mM concentration ribulosebisphosphate offered 30–40% protection against rose bengal-sensitized photooxidation of the enzyme.

A partially inactivated enzyme with 30% residual activity was fully sensitive to positive effectors such as NADPH, phosphogluconate and P_i. These are known positive effectors of the enzyme at low HCO_3^- concentrations in the presence of Mg^{2+} [19,20]. When the partially inactivated enzyme was preincubated with these effectors, the observed activation was 1.4-fold with NADPH, 1.8-fold with phosphogluconate and 2-fold with 5 mM P_i. The enzyme kept in dark containing the same amount of dye showed a similar activation pattern with these effectors. These results suggest that photooxidation did not alter the regulatory interaction of the enzyme with these effectors.

Chemical modification using diethylpyrocarbonate. Diethylpyrocarbonate at slightly acidic pH values has been shown to modify histidine residues in proteins with considerable specificity [21]. This reagent was also used in the studies to show that histidine residues are essential for activity in the case of ribulosebisphosphate carboxylase/oxygenase.

The enzyme rapidly lost its activity when incubated in the presence of diethylpyrocarbonate at 25°C at pH 6.1. The inactivation followed pseudo-first-order reaction kinetics. The time course of inactivation of the enzyme at different diethylpyro-

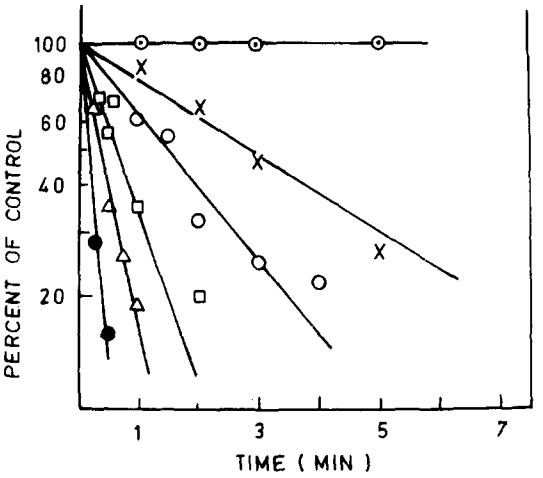

Fig. 5. Time course of inactivation of the enzyme at different diethylpyrocarbonate concentrations. The enzyme in 50 mM Hepes-KOH (pH 6.1)/10 mM $NaHCO_3$/20 mM $MgCl_2$ was incubated with the indicated concentrations of diethylpyrocarbonate. At the indicated time intervals aliquots were removed and quenched in a buffer system containing 20 mM histidine. Enzyme containing an equal amount of ethanol was taken as 100%. Ethanol had no effect on the enzyme. ⊙———⊙, 0 mM; X———X, 0.5 mM; ○———○, 1 mM; □———□, 2 mM; △———△, 3 mM and ●———●, 5 mM.

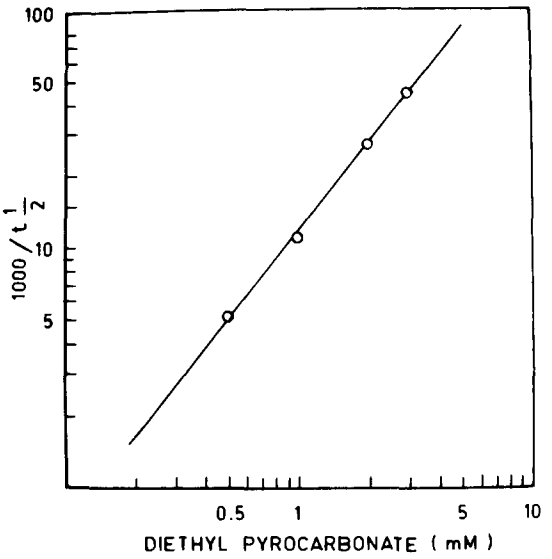

Fig. 6. Log [$1000/t_{1/2}$ (s)] against log (diethylpyrocarbonate) plot of the data in Fig. 5. n = 1.22.

carbonate concentrations is shown in Fig. 5. The order of reaction with respect to diethylpyrocarbonate was determined according to the method employed by several workers [22–25]. In this type of plot a straight line is expected with a slope equal to n, where n is the number of inhibitor molecules reacting with each active (protomeric) unit to produce inactive enzyme-inhibitor complex [22–24]. When the data of Fig. 5 were plotted on this basis, an n value of 1.22 was obtained (Fig. 6). Ribulosebisphosphate carboxylase from spinach, as mentioned earlier, is composed of eight protomeric or active units of 70000 daltons each [31], and since the n value refers to the reaction of an inhibitor molecule with each active unit of an enzyme, it is concluded that on an average one diethylpyrocarbonate molecule was bound to each 70000-dalton protomeric unit when inactivation occurred.

Relation between activity and number of histidine residues modified. The extent of modification of histidine residues by diethylpyrocarbonate was conveniently determined by absorbance maxima in difference spectra [26]. The data presented in Fig. 7A show a characteristic absorbance band having a peak at 240 nm following the reaction of diethylpyrocarbonate with ribulosebisphosphate carboxylase.

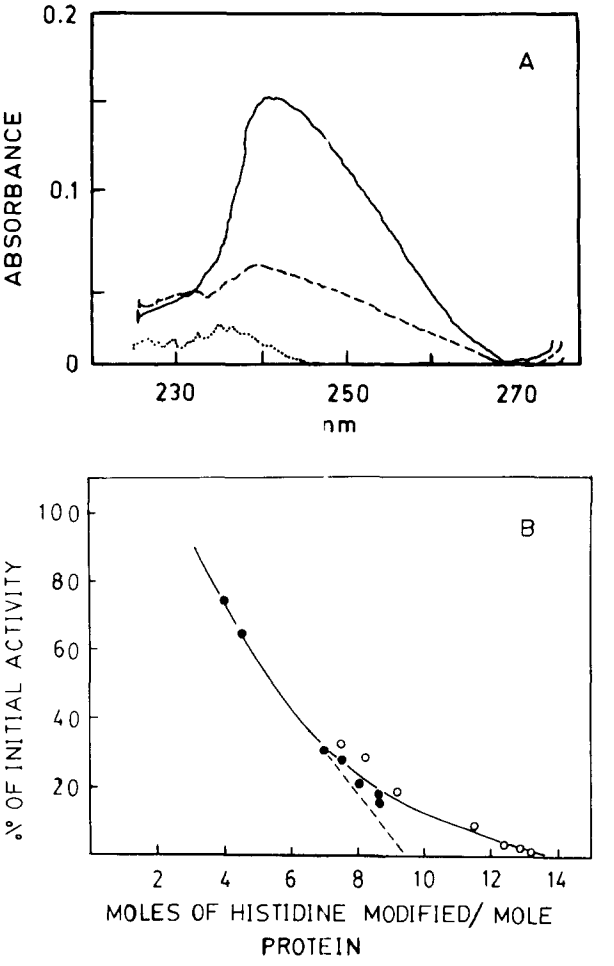

Fig. 7A. Difference spectra of diethylpyrocarbonate-modified enzyme. The spectra were run at different time intervals after addition of diethylpyrocarbonate to the sample cuvette. The reference cell contained all ingredients except diethylpyrocarbonate but had an equal amount of ethanol. At the end of 45 min 0.4 M NH_2OH was added to both sample and reference cuvettes and spectra recorded at several time intervals. Solid line, spectrum taken 45 min after diethylpyrocarbonate addition. Broken line, spectrum taken 30 min after NH_2OH addition to the above sample. Dotted line is taken as base line for calculation. B. Relation between the number of histidine residue modified with percent of initial activity remaining. The enzyme (2.9 μM) was incubated with 0.25 and 0.5 mM diethylpyrocarbonate in tricine-NaOH buffer (pH 7.0)/ 10 mM $NaHCO_3$/20 mM $MgCl_2$. Difference spectra run at several time intervals after diethylpyrocarbonate treatment of the enzyme. At each time interval an aliquot was removed and the enzyme activity determined. The data of two different experiments done at two different diethylpyrocarbonate concentrations are shown here in different symbols.

The relation between the activity and number of histidine residues modified calculated from the absorbance at 240 nm is depicted in Fig. 7B. The complete loss of enzyme activity resulted from the modification of 9.5 histidine residues per 557 000-dalton holoenzyme. In other words, per 70 000-dalton combination of large and small subunit, modification of 1.18 histidine residues caused complete loss of enzyme activity. This is in agreement with the data on kinetics of inactivation (Fig. 6).

Reversal of diethylpyrocarbonate inhibition by NH_2OH. Hydroxylamine at 0.4 M concentration caused a considerable reversal of diethylpyrocarbonate inhibition with a concomitant decrease in absorbance at 240 nm indicating a nucleophilic attack on *N*-carbethoxyhistidine (Fig. 7A). The modified enzyme having 30 and 48% residual activity was reactivated to 78 and 85%, respectively, following 16 h treatment with 0.4 M NH_2OH. The diethylpyrocarbonate-modified enzyme in the absence of NH_2OH did not show any increase in residual activity.

Specificity of diethylpyrocarbonate inactivation. Diethylpyrocarbonate is also known to react with amino acids such as cysteine and lysine in a model system. The formation of *N*-acetyltyrosine has only been rarely reported [16,21]. It therefore became necessary to eliminate the possibility of the inhibition being due to the modification of the residues other than histidine.

Tyrosine, sulfhydryl and lysine groups. No decrease in the difference absorbance was observed at 278 nm in the case of diethylpyrocarbonate-modified enzyme (data not shown). The number of total titratable -SH groups of the enzyme with and without diethylpyrocarbonate modification were found to be the same under both native and denaturing conditions (8 M urea), indicating that -SH groups were still available for modification even after diethylpyrocarbonate treatment. After protecting the essential lysine groups by pyridoxal phosphate the subsequent modification of the enzyme by diethylpyrocarbonate showed that the site of action of diethylpyrocarbonate is not lysine residues (Table I), moreover the number of lysine groups of the enzyme reacting with pyridoxal phosphate with and without diethylpyrocarbonate modification were found to be the same (19 residues modified/mol enzyme).

Protection of the enzyme activity against diethylpyrocarbonate inactivation. The results presented so far indicate that the inactivation of the enzyme by diethylpyrocarbonate is due to the modification of essential histidine residues. Substrate (ribulosebisphosphate) and several other effectors of the enzyme offered substantial protection against diethylpyrocarbonate inactivation (Table II). Various ligands used to prevent diethylpyrocarbonate inactivation had no effect on the formation of *N*-carbethoxyimidazole in a model system.

TABLE I

EFFECT OF PYRIDOXAL PHOSPHATE ON DIETHYLPYROCARBONATE INACTIVATION OF RIBULOSEBISPHOSPHATE CARBOXYLASE ACTIVITY

Diethylpyrocarbonate inactivation was done as described in Materials and Methods. Samples 3–6 were first preincubated with 1 mM pyridoxal phosphate in the dark for 10 min. To samples 4 and 5 after a 10 min period 1 mM diethylpyrocarbonate was added. They were preincubated in the dark for an additional 10 min. To samples 5 to 8 were added 10 mM NH_2OH and they were preincubated for another 2–3 min. All samples after activation were assayed for oxygenase and carboxylase activity. All control without diethylpyrocarbonate had an equal amount of ethanol. The enzyme used in all the experiments had a specific activity of 1.5 units/mg protein and 0.1 units/mg protein for carboxylase and oxygenase, respectively.

Preincubation conditions	Percent activity remaining
1. None	100
2. Diethylpyrocarbonate (1 mM) 10 min	13
3. Pyridoxal phosphate (1 mM) 10 min (dark)	2
4. Pyridoxal phosphate + diethylpyrocarbonate	0
5. Pyridoxal phosphate + diethylpyrocarbonate + NH_2OH	20
6. Pyridoxal phosphate + NH_2OH	90
7. NH_2OH	90
8. Diethylpyrocarbonate + NH_2OH	10

TABLE II

PROTECTION OF SPINACH RIBULOSEBISPHOSPHATE CARBOXYLASE/OXYGENASE AGAINST INACTIVATION BY DIETHYLPYROCARBONATE

The percent activity remaining was compared with control lacking diethylpyrocarbonate but contained the same amounts of protective agents and ethanol. Diethylpyrocarbonate concentration was 1 mM in all the experiments. All the samples were reactivated with HCO_3^- and Mg^{2+} after diethylpyrocarbonate treatment and before assay.

Additions	Concentration (mM)	% Activity remaining after 10 min incubation	
		Carboxylase	Oxygenase
None	–	100	100
Diethylpyrocarbonate	1	6	8
Ribulosebisphosphate + diethylpyrocarbonate	2	55	42
Fructose 1,6-bisphosphate + diethylpyrocarbonate	2.5	51	54
Phosphoglyceric acid + diethylpyrocarbonate	5	50	50
Phosphate + diethylpyrocarbonate	20	62	65
Ribose 5-phosphate + diethylpyrocarbonate	2.5	6	8

Discussion

Histidine residues have been implicated in the catalytic mechanism of several enzymes [16,18, 25–27]. Our data show that ribulosebisphosphate carboxylase/oxygenase was rapidly inactivated by two histidine-selective reagents.

Even though rose bengal offers greater specificity for histidine at the active site [26], photooxidation of other amino acid residues like methionine, tyrosine, cysteine and tryptophan is possible. We had routinely used dithiothreitol and thioglycollate to reduce protein after photooxidation, and since the oxidation products of methionine and cysteine can be reduced by these reducing agents, the possibility of the inhibition being due to oxidation of methionine or cysteine may not exist. No modification of tyrosine residues was observed as indicated from the spectral analysis of the photooxidised enzyme. The intrinsic fluorescence intensity of the protein after photooxidation was not found to be any different in the time periods used in these studies. This observation indicated that tryptophan residues were not destroyed by photooxidation. Given these observations, we may conclude that the inhibition of the enzyme activity by photooxidation was due to the modification of critical histidine residues of the protein.

Our data on the inactivation of the enzyme by diethylpyrocarbonate show that the loss of enzyme activity was due to the modification of histidine residues at or near the active site. This interpretation is supported by following observations. (1) At pH 6.1 modification by diethylpyrocarbonate is highly specific for histidine residues. (2) A direct correlation was observed between the number of histidine residues modified with the rate of inactivation of the enzyme. (3) Sulfhydryl, lysine and tyrosine residues were not modified by diethylpyrocarbonate treatment. (4) Decarbethoxylation by NH_2OH also showed that amino groups were not modified. (5) Substrate and other effectors substantially prevented inactivation of the enzyme indicating that diethylpyrocarbonate was interacting at or near the active site. (6) The inhibition was faster at pH values of 6.2–6.6 as compared to pH 8.0 [27].

Diethylpyrocarbonate has been shown to react specifically with a single histidine residue in certain proteins (class A), in other cases the modification of activity has been correlated with the modification of one or more histidine residues despite possible modification of other residues (class B). Our data discussed here show that the enzyme ribulosebisphosphate carboxylase belongs to class A according to the classification of Miles [16].

Tyrosine residues have so far not been shown as essential for activity in the case of ribulosebisphosphate carboxylase. *O*-Carbethoxy-*N*-acetyltyrosine

ethyl ester is 4-times less reactive with NH_2OH than carbethoxyimidazole [16]. These observations along with the results presented in this paper indicate that diethylpyrocarbonate inactivation of the enzyme may not be due to the modification of tyrosine residues. *N*-Acetylcysteine is quite unstable as compared to *N*-carbethoxyimidazole and also the number of titratable -SH groups were the same as control in the case of the diethylpyrocarbonate-modified enzyme suggesting that diethylpyrocarbonate treatment did not modify -SH groups of the enzyme under the experimental conditions used in these studies.

It has been shown by several workers that 16 amino groups of lysine are essential for activity of ribulosebisphosphate carboxylase [8,28,29]. These lysine residues from Schiff base with pyridoxal phosphate. This observation was used in protecting the essential lysine residues during diethylpyrocarbonate modification. The pyridoxal phosphate inhibition is rapidly reversed by NH_2OH, however, the pyridoxal phosphate-protected enzyme after diethylpyrocarbonate treatment could not be reactivated with NH_2OH. This suggested that the site of action of diethylpyrocarbonate was other than the essential lysine residues. Since the total titratable lysine residues were the same as control even after diethylpyrocarbonate modification of the enzyme and as these residues fall in the domain of the active site of the enzyme, particularly the binding sites of CO_2 and ribulosebisphosphate, we believe that diethylpyrocarbonate modification does not affect ribulosebisphosphate or CO_2 binding sites.

Saluja and McFadden [27] have indicated the presence of histidine residues at the active site of ribulosebisphosphate carboxylase. They have suggsted that inactivation of the enzyme results from modification of 2–3 histidine residues per 70 000-dalton combination of large and small subunit. These workers had used Tris-HCl buffer for the modification studies. It is known that Tris-HCl accelerates the decomposition of diethylpyrocarbonate and also reacts with it as a nucleophile. Inorganic phosphate has been shown to prevent diethylpyrocarbonate inactivation of the enzyme (Table II), this is another buffer system used by these workers for the correlation studies.

In the present studies we used two chemicals to modify the enzyme. The correlation of the activity with number of histidines modified, determined by two different methods, showed that inactivation resulted from the modification of a single critical histidine residue per 70 000-dalton combination of large and small subunit.

Paech et al. [28,29], have indicated a possibility that lysine or sulfhydryl groups may be involved in proton transfer in the reaction mechanism of ribulosebisphosphate carboxylase/oxygenase. The evidence presented in this paper suggests that histidine residues may be essential for catalysis making them a better candidate as a basic group which would abstract protons at C-3 of ribulosebisphosphate to initiate enediole formation.

References

1 Jensen, R.G. and Bahr, J.T. (1977) Ann. Rev. Plant Physiol. 28, 379–400
2 Quayle, J.R., Fuller, R.C., Benson, A.A. and Calvin, M. (1954) J. Am. Chem. Soc. 76, 3610
3 Weissbach, A., Smyrnoitis, P.Z. and Horecker, B.L. (1954) J. Am. Chem. Soc. 76, 3611
4 Bowes, G., Ogren, W.L. and Hageman, R.H. (1971) Biochem. Biophys. Res. Commun. 45, 716–722
5 Paulsen, J.M. and Lane, M.D. (1966) Biochemistry 5, 2350–2357
6 Rabin, B.R. and Trown, P.W. (1964) Nature (Lond.) 202, 1290
7 Lane, M.D. and Miziorko, H.M. (1978) in Photosynthetic Carbon Assimilation (Siegelman, H.W. and Hind, G., eds.), Basic Sciences, Vol. 11, pp. 19–40, Plenum Press, New York
8 Schloss, J.V., Norton, L., Stringer, C.D. and Hartman, F.C. (1978) Biochemistry 17, 5620–5631
9 Paech, C., Ryan, F.J. and Tolbert, N.E. (1977) Arch. Biochem. Biophys. 179, 279–288
10 Whitman, W.B. and Tabita, F.R. (1978) Biochemistry 17, 1282–1287
11 Sugiyama, T. and Akazawa, T. (1967) J. Biochem. (Tokyo) 62, 474–482
12 Purohit, K., McFadden, B.A. and Lawlis, V.B. (1979) Arch. Microbiol. 121, 75–82
13 Lawlis, V.B. and McFadden, B.A. (1978) Biochem. Biophys. Res. Commun. 80, 580–585
14 Wishnick, M. and Lane, M.D. (1971) Methods Enzymol. 23, 570–577
15 Lorimer, G.H., Badger, M.R. and Andrews, T.J. (1977) Anal. Biochem. 78, 66–75
16 Miles, E.W. (1977) Methods Enzymol. 47, 431–442
17 Ellman, G.L. (1959) Arch. Biochem. Biophys. 82, 70–77
18 Rippa, M. and Pontremoli, S. (1968) Biochemistry 7, 1514–1518
19 Buchanan, B.B. and Schurman, P. (1973) J. Biol. Chem. 248, 4956–4962

20 Bhagwat, A.S. (1981) Plant Sci. Letts. in the press
21 Melchior, W.B. and Fahrney, D. (1970) Biochemistry 9, 251–258
22 Keech, D.B. and Farrant, R.K. (1968) Biochim. Biophys. Acta 151, 493–503
23 Levy, H.M., Leber, P.D. and Ryan, E.M. (1963) J. Biol. Chem. 238, 3654–3659
24 Hollenberg, P.F., Flashner, M. and Coon, M.J. (1971) J. Biol. Chem. 246, 946–953
25 George, A.L. and Borders, C.L. (1979) Biochim. Biophys. Acta 569, 63–69
26 Westhead, E.W. (1965) Biochemistry 4, 2139–2144
27 Saluja, A.K. and McFadden, B.A. (1980) Biochem. Biophys. Res. Commun. 94, 1091–1097
28 Paech, C. and Tolbert, N.E. (1978) J. Biol. Chem. 253, 7864–7873
29 Paech, C., McCurry, S.D., Pierce, J. and Tolbert, N.E. (1978) in Photosynthetic Carbon Assimilation (Siegelman, H.W. and Hind, G., eds.) Basic Sciences Vol. 11, pp. 227–243, Plenum Press, New York
30 Paech, C., Ryan, F.J. and Tolbert, N.E. (1977) Arch. Biochem. Biophys. 179, 279–288
31 Nishimura, M. and Akazawa, T. (1973) Biochem. Biophys. Res. Commun. 54, 842–848