Specific and Irreversible Inactivation of Pepsin by Substrate-like Epoxides*

(Received for publication, January 26, 1971)

JORDAN TANG‡

From the Oklahoma Medical Research Foundation and the Department of Biochemistry and Molecular Biology, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma 73104

SUMMARY

Several substrate-like epoxides were found to act as specific and irreversible inactivators of pepsin. Of these, 1,2-epoxy-3-(p-nitrophenoxy) propane (EPNP) was the most potent. When EPNP reacted with pepsin, all enzyme activity was lost. Two molecules of EPNP were found to be covalently bound to each molecule of completely inactivated enzyme. In this reaction, two carboxyl groups of the enzyme are each apparently esterified by one hydroxyl group of glycol formed from EPNP. The esterification of carboxylates by epoxides is a well established reaction. The modified enzyme, EPNP-pepsin, released the bound EPNP groups in alkaline solution. On thin layer chromatography, the released modifying group was identified as the glycol of EPNP. These observations support the view that esterification was the inactivation reaction.

Prior reaction of pepsin with diazoacetyl-DL-norleucine methyl ester or p-bromophenacyl bromide was still followed by the reaction of 2 EPNP residues per pepsin molecule. The EPNP-reactive carboxyl group must, therefore, differ from those carboxyl groups reacted with diazoacetyl-DL-norleucine methyl ester and p-bromophenacyl bromide. EPNP failed to inactivate pepsinogen. Moreover, in the presence of synthetic substrates or their hydrolytic products, the inactivation of pepsin by EPNP was significantly slower. Results on the EPNP incorporation in the presence and absence of substrates indicated that only one of the two modified carboxyl groups was protected by the presence of the dipeptide substrate. This “active center carboxyl group” may participate in the catalysis of the enzyme. The second EPNP-reactive carboxyl group was apparently not essential for enzyme activity.

EPNP was found to inactivate other gastric proteases: human gastricsin, human pepsin, and bovine rennin. The stoichiometry of the reaction in human gastricsin and pepsin was identical with that in porcine pepsin.

In the past few years, several specific inhibitors have been found for pepsin. Detailed investigation of their effects on

* This work was supported by Research Grant AM 01107 and AM-66487 from the National Institutes of Health.
‡ Recipient of Career Development Award 5-K04-AM-38638.
which were structurally analogous to some amino acid residues, such as phenylalanine, known to have an affinity for pepsin. The chemical structures are compared in Scheme 1.

Several epoxides were found to inactivate pepsin irreversibly, apparently by esterifying two carboxyl groups, one of which is in the active center. Neither of these carboxyl groups had been modified previously.

EXPERIMENTAL PROCEDURE

Materials

Porcine pepsin (three times crystallized) and bovine hemoglobin substrate were obtained from Pentex, porcine pepsinogen from Worthington, and crystalline rennin from Nutritional Biochemicals. Human gastrin and pepsin were prepared as described previously (22, 23).

Diazoacetyl-11-norleucine methyl ester was provided by Dr. John Mills, Oklahoma Baptist University, and was synthesized as described by Rajagopalan, Stein, and Moore (2). 1,2-Epoxyethyl benzene, 1,2-epoxy-3-phenoxynaphthylene, 1,2-epoxy-3-(p-nitrophenoxy) propane, and 1,2-bromophenacyl bromide were obtained from Eastman Organic Chemicals. Propylene oxide and 1,2-epoxy butane were obtained from Aldrich. The polyamide thin layer chromatographic layers were obtained from Gallard-Schlesinger. Other chemicals, which were of commercial sources, were of the highest purity available and were used without further purification.

Methods

Proteolytic Activity—The proteolytic activity was measured with bovine hemoglobin used as substrate. The procedure was essentially that of Anson and Mirsky (24), described previously (25).

Milk-clotting Activity—The procedure of Berriedge was followed for the determination of milk-clotting activity (26).

Inhibition Studies—A typical reaction mixture consisted of 0.5 ml of 0.1% solution of pepsin in 0.1 M sodium citrate buffer of appropriate pH. To this solution, about 0.1 ml of EB or EPP was added. In the case of EPNP, 5 mg of solid powder were added, and the solution was incubated at room temperature (25° ± 1°C). Since the solubility of the epoxy compounds was relatively low, a rotating mixer was used to keep the solution stirred continuously, thereby maintaining a state of saturation. At different time intervals, 10-μl aliquots were withdrawn for the assay of enzyme activity.

Determination of Pepsin Inactivation and EPNP Incorporation in Presence of Peptide Substrates—Inactivation experiments were performed with 10 ml of 0.1% enzyme solution in 0.1 M sodium citrate buffer. The conditions for the control experiments were identical with those described above. In experiments to test the effect of synthetic peptide substrates, either Z-L-alanyl-L-tyrosine (7 × 10⁻⁴ M) or glycy1-L-phenylalanyl amide (6.82 × 10⁻² M) was included in the reaction solution. After initial mixing of the solution, 0.1-ml aliquots were withdrawn at 135, 300, and 615 min for determination of enzyme activity. Separate 2.5-ml aliquots were taken at the same time intervals. These samples were quickly cooled in an ice bath and then filtered, and the filtrates were dialyzed in a cold room with vigorous stirring against 0.1 M sodium acetate buffer, pH 5.0. After five changes of dialysate buffer, samples were analyzed spectrophotometrically for EPNP content (see below).

Preparation of EPNP-Pepsin—Crystalline pepsin (100 mg) was reacted in 50 ml of 0.1 M sodium citrate buffer, pH 4.6, with a suspension of about 10 mg of EPNP. After the solution had stood for 72 hours at room temperature, it was dialyzed for 40 hours against five changes of 0.001 M acetic acid and then stored at 4°C, or, in an alternative procedure, 10 ml of reaction mixture were chromatographed on a Sephadex G-25 column (2 × 62 cm) eluted with 0.005 M acetic acid. EPNP-pepsin was eluted in a peak at 90 ml of the elution volume. EPNP-OH, the hydrolytic product of EPNP, appeared at 300 ml of the eluent.

Reaction of Pepsin and Diazoacetyl-11-norleucine Methyl Ester—Pepsin was reacted with diazoacetyl-11-norleucine methyl ester according to the procedure of Rajagopalan et al. (2). The reaction mixture was dialyzed in the cold against distilled water. The treated pepsin had no proteolytic activity, and 1.3 residues of norleucine were found.

Preparation of p-Bromophenacyl Bromide-reacted Pepsin—Pepsin was reacted with p-bromophenacyl bromide by the method of Ehrunger et al. (14). The reacted pepsin was dialyzed in the cold against 0.001 M acetic acid.

Spectrophotometric Determination of EPNP in EPNP-Pepsin—The content of EPNP in EPNP-pepsin was calculated from the extinction coefficients which were experimentally determined (see “Preparation of EPNP-OH” in this section and “Stoichiometry” under “Results”). The molar extinction coefficient of pepsin at 280 μM was experimentally determined to be 46,000. The optical density of EPNP-pepsin in 0.001 M acetic acid was determined spectrophotometrically at 280 and 315 μM. The equation used for calculation was:

\[
\text{No. of EPNP residues per molecule of pepsin} = \frac{\text{OD}_{280} \times 4.16}{\text{OD}_{315} \times 0.438}
\]

Preparation of EPNP-OH—EPNP (12 mg) was placed in a 100-ml graduated flask to which 10 ml of 0.05 M acetic acid were added. The solution was heated in an oven at 45°C until all solid EPNP had dissolved (about 2 days). The solution was then diluted with distilled water to a total volume of 100 ml. Ross (21) showed that, under such conditions, the epoxides are converted mostly to glycols without significant formation of ester...
with acetic acid. The absorption spectrum of this solution (see "Results") yields a major extinction of EPNP-OH of 11,000 at 315 m\(\mu\) and 4,960 at 280 m\(\mu\).

**Amino Acid Analysis**—The protein was hydrolyzed in 6 M HCl in a sealed, evacuated tube at 105° for 24 hours. Amino acids were determined with a Spinco model 120-B amino acid analyzer, with a modified "range card" in the recorder to permit quantitative analysis in the range of 0.001 to 0.1 \(\mu\) mole of amino acids. Analysis was carried out according to the procedure of Spackman (27).

---

**FIG. 1**. Inactivation of pepsin by epoxides. The reaction solution contained 1 mg of pepsin in 1 ml of 0.1 M sodium citrate buffer, pH 4.6. Excess epoxide compounds were added and the solutions were shaken at room temperature. Aliquots were withdrawn and assayed for proteolytic activity, with hemoglobin used as substrate.

**TABLE I**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Concentration</th>
<th>Activity</th>
<th>Inhibition</th>
<th>Maximum inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPNP</td>
<td>3.21 \times 10^{-3}</td>
<td>51.6</td>
<td>48.4</td>
<td>100</td>
</tr>
<tr>
<td>EPNP-OH</td>
<td>4.7 \times 10^{-3}</td>
<td>97.0</td>
<td>3.0</td>
<td>6.2</td>
</tr>
<tr>
<td>EPNP + EPNP-OH</td>
<td>Same as above</td>
<td>54.9</td>
<td>45.1</td>
<td>83</td>
</tr>
<tr>
<td>Z-Ala-Tyr</td>
<td>7.0 \times 10^{-4}</td>
<td>61.0</td>
<td>38.0</td>
<td>78.5</td>
</tr>
<tr>
<td>Z-Glu-Tyr</td>
<td>7.0 \times 10^{-2}</td>
<td>76.2</td>
<td>23.8</td>
<td>49.1</td>
</tr>
<tr>
<td>Gly-Phe amide</td>
<td>6.82 \times 10^{-2}</td>
<td>89.6</td>
<td>10.4</td>
<td>21.5</td>
</tr>
<tr>
<td>Tyr-Tyr</td>
<td>1.0 \times 10^{-3}</td>
<td>77</td>
<td>23</td>
<td>47.5</td>
</tr>
<tr>
<td>Acetyl-Leu</td>
<td>2.0 \times 10^{-3}</td>
<td>53.5</td>
<td>46.5</td>
<td>96.1</td>
</tr>
<tr>
<td>Z-Glu</td>
<td>4.0 \times 10^{-3}</td>
<td>61.3</td>
<td>38.7</td>
<td>80</td>
</tr>
</tbody>
</table>

---

**RESULTS**

**Inactivation of Pepsin by Epoxides**—The course of inactivation is shown in Fig. 1. After 70 hours, both EB and EPP had inactivated the enzyme completely. For EPNP, an additional 20 to 40 hours were necessary for complete inactivation. The inactivated pepsin did not regain its activity after thorough dialysis in the cold against 0.001 M acetic acid, indicating that loss of enzymic activity was not due to the competitive inhibition of glycyls produced in the spontaneous hydrolysis of epoxy compounds. The incubation of pepsin with EPNP-OH resulted in only minor loss of activity (Table I). Therefore, the epoxides, and not their hydrolytic products, must have been responsible for inactivation. Inactivation was carried out in acid solutions at four different pH values: 5.5, 4.6, 3.5, and 2.2. In all four solutions, the inactivation proceeded in similar fashion. The use of citrate or acetate buffer had no apparent effect.

The apparent second order inactivation constant was calculated for the epoxides shown in Fig. 1 and for two additional compounds, propylene oxide and 1,2-epoxybutane (Table II). A comparison of the structures of these epoxides and their apparent inactivation constants made it clear that the reactivity of epoxides was related to their structural similarity to a pepsin substrate. EPNP was the most potent inactivator, with a \(k_{\text{app}}\) value approximately 23 times that of propylene oxide. This is apparently an effect of affinity labeling. The relatively slower inactivation rate by EPNP in Fig. 1 was evidently due to its low solubility.

In order to investigate the specificity of this inactivation, porcine pepsinogen was incubated with the epoxides in 0.1 M sodium acetate buffer, pH 5.5, under the same conditions used in the pepsin experiments. Aliquots were taken and assayed for activity after acidification. EPP and EPNP had no effect on pepsinogen activity after 24 hours of incubation. EB inactivated pepsinogen only slightly, i.e., at approximately 10% of the rate at which it inactivated pepsin. Although this phenomenon was not investigated further, it seemed clear that the epoxide inactivation was essentially specific for pepsin.

The reaction mixture of pepsin and EPNP was passed through a Sephadex G-25 column to separate pepsin from small molecular compounds. As shown in Fig. 2, the pepsin peak (monitored by absorption at 280 m\(\mu\)) showed a definite absorption at 315 m\(\mu\), which is the absorption maximum of EPNP-OH (see "Stoichiometry" below). The ratio of OD\(280:OD\(315\)) was calculated to be 0.49 and was constant in the different fractions throughout the pepsin peak. The result indicated that EPNP possibly reacted covalently to pepsin. The amino acid composition of EPNP-pepsin did not differ significantly from that of the native pepsin. It was found, however, that the content of EPNP in pepsin could be measured spectrophotometrically (see below). Consequently, further experiments were carried out with this compound.

**Stoichiometry of Reaction of EPNP with Pepsin**—The absorption spectra of EPNP-OH, pepsin, and EPNP-pepsin are shown in Fig. 3. EPNP-OH showed a maximum absorption at 315 m\(\mu\). The increased absorbance of EPNP-pepsin in the wave length region of 310 to 400 m\(\mu\) (compared to that of pepsin) was apparently contributed by pepsin bound EPNP. When the number of EPNP molecules bound to each molecule of pepsin was calculated (see "Methods"), essentially 2 residues of...
The apparent inactivation constant, $k_{app}$, was calculated on the basis of a second order reaction, by using the equation: $k_{app} = \frac{v_i}{[E][I]}$, where $[E]$ and $[I]$ are the concentrations of enzyme and inactivator, respectively. The initial inactivation velocity, $v_i$, is expressed in moles per liter per hour. The inactivation solution contained 1 mg of pepsin per ml of 0.1 M acetic acid-HCl buffer, pH 3.0. The epoxide concentrations were, in the same order listed in the table, 0.47 M, 0.38 M, $2.34 \times 10^{-5}$ M, $1.66 \times 10^{-5}$ M, and $3 \times 10^{-5}$ M, respectively. The inactivation was carried out at 25° and aliquots of 10 μl were taken at 30, 60, and 180 min and assayed for proteolytic activity. The initial, near linear portion of the curves was taken for the calculation of $v_i$.

<table>
<thead>
<tr>
<th>Epoxide</th>
<th>Structure</th>
<th>Homologous amino acid residue</th>
<th>$k_{app}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO</td>
<td>CH₂-CH-CH₂</td>
<td>Glycyl</td>
<td>5.8</td>
</tr>
<tr>
<td>EB</td>
<td>CH₂-CH₁-CH₂</td>
<td>Alanyl</td>
<td>13.8</td>
</tr>
<tr>
<td>EB</td>
<td>CH₂-CH₁-CH₂</td>
<td>Valyl or phenylalanl</td>
<td>25.7</td>
</tr>
<tr>
<td>EPP</td>
<td>CH₂-CH₁-CH₂</td>
<td>Phenylalanl</td>
<td>25.3</td>
</tr>
<tr>
<td>EPNP</td>
<td>NO₂-CH₂-CH₁-CH₂</td>
<td>p-Nitrophenylalanl</td>
<td>133.3</td>
</tr>
</tbody>
</table>

* PO, propylene oxide.
* EB, 1,2-epoxy butane.

EPNP were bound to each molecule of pepsin (Table I). Inactivation experiments carried out at pH 4.6, 3.5, and 2.2 produced the same results.

A separate method was used to determine the stoichiometry of the reaction of EPNP with pepsin. It was found that EPNP could be released from pepsin by incubating EPNP-pepsin in an alkaline solution (see "Stability"). Therefore, EPNP-pepsin, obtained from Sephadex G-25 chromatography (Fig. 2), was incubated in a solution at pH 10.4, 45°, for 72 hours. The solution was then chromatographed on a Sephadex G-25 column. As shown in Fig. 4, the pepsin peak contained only trace absorbance at 315 μm. The second peak, which appeared at the same position as EPNP-OH, had the characteristic high absorbance, 315 μm, of this compound. The quantitative ratio of pepsin and EPNP-OH eluted from these two peaks was found to be 1:1.45, thus confirming the finding from direct spectrophotometric determination.

As mentioned in the introductory section, diazoacetyl-
norleucine methyl ester and p-bromophenacyl bromide are known to react with two separate carboxylic groups, one in and the other near the active center of pepsin. To determine whether EPNP would react with the same carboxylic groups, the following experiments were carried out. Pepsin was reacted separately with diazooctetyl-norleucine methyl ester and p-bromophenacyl bromide and then allowed to react with EPNP for 70 hours at room temperature. After thorough dialysis, the content of EPNP in pepsin was determined spectrophotometrically. As shown in Table III, 1.92 residues of EPNP were found in pepsin treated with diazoinactivator. When this protein was subjected to amino acid analysis, 1.3 residues of norleucine were found, as was the case before EPNP reaction. When EPNP-inactivated, dialyzed pepsin was reacted with diazooctetyl-norleucine methyl ester, 1.2 residues of norleucine were incorporated. These results indicated that the incorporation of 2 residues of EPNP was probably not due to a replacement of norleucine-containing inactivator. Therefore, these inactivators must have reacted with different sites. When pepsin was treated with p-bromophenacyl bromide, 1.98 residues of EPNP were found to have reacted, indicating that the sites reactive with EPNP must differ from those reacted with other inactivators.

Identification of Hydrolytic Product of EPNP-Pepsin—If the inhibition of pepsin were due to the esterification, the hydrolytic products of EPNP-pepsin would be EPNP-OH and pepsin. Consequently, the chromatographically isolated product which had been separated from EPNP-pepsin after treatment in alkaline solution (second peak in Fig. 4) was subjected to thin layer chromatography on polyamide layers. As shown in Fig. 5, the hydrolytic product of EPNP-pepsin migrated to the identical position as EPNP-OH in two different solvent systems.

Table III

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>pH of inactivation by EPNP</th>
<th>Preliminary reaction with other inactivator</th>
<th>No. of EPNP molecules bound per molecule of enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin (porcine)</td>
<td>4.6</td>
<td>None</td>
<td>1.89</td>
</tr>
<tr>
<td>Pepsin (porcine)</td>
<td>3.3</td>
<td>None</td>
<td>1.92</td>
</tr>
<tr>
<td>Pepsin (porcine)</td>
<td>2.2</td>
<td>None</td>
<td>1.85</td>
</tr>
<tr>
<td>Pepsin (porcine)</td>
<td>3.5</td>
<td>Diazooctetyl-norleucine methyl ester</td>
<td>1.92</td>
</tr>
<tr>
<td>Pepsin (porcine)</td>
<td>3.5</td>
<td>p-Bromophenacyl bromide</td>
<td>1.98</td>
</tr>
<tr>
<td>Gastricin (human)</td>
<td>4.6</td>
<td>None</td>
<td>1.97</td>
</tr>
<tr>
<td>Pepsin (human)</td>
<td>4.6</td>
<td>None</td>
<td>2.02</td>
</tr>
<tr>
<td>Rennin (bovine)</td>
<td>4.6</td>
<td>None</td>
<td>4.20</td>
</tr>
</tbody>
</table>

* a The amount of EPNP in pepsin was estimated after alkaline hydrolysis (pH 10.5, 50°C, for 48 hours) and separation of a column of Sephadex G-25 (see “Stoichiometry,” under “Results”). Since the p-bromophenacyl group contributes to absorption at 280 mμ, a direct spectrophotometric measurement gave a value of 1.3 residues of EPNP per pepsin molecule.

Fig. 5. Thin layer chromatographic pattern of EPNP, EPNP-OH, and pepsin-bound EPNP-OH after hydrolysis from the enzyme by alkali. The latter material (Peak 2) was obtained from the second peak in Fig. 4. A was developed in a water solution containing 1.5% formic acid. B was developed in a solvent of benzene and glacial acetic acid (9:1).

Fig. 6. The loss of EPNP group from EPNP-pepsin during incubation in alkaline solutions. Aliquots of 1 ml containing 5 mg of EPNP-pepsin were taken at different time intervals. The samples were chromatographed on a column of Sephadex G-25 as described in Fig. 4. The content of EPNP in pepsin was determined spectrophotometrically.
incorporation (or 2 residues of EPNP incorporation at 100% inactivation). In the absence of substrate, the curve representing the rate of EPNP incorporation was only slightly higher than that representing the rate of pepsin inactivation. This indicated that the relationship of the incorporation of about 2 residues of EPNP for the inactivation of each enzyme molecule (Table III) remained consistent during the course of the reaction. The presence of either of the synthetic dipeptides, Z-L-alanyl-L-tyrosine or glycyl-L-phenylalanine amide (6.82 X 10^-2 M) had a like, but more pronounced effect. (Abscissa on left represents pepsin inactivation, and on right, EPNP incorporation.)

![Fig. 7. The effect of synthetic dipeptides on the rate of pepsin inactivation by EPNP and on the rate of incorporation of EPNP into the enzyme. In the absence of synthetic peptides, the rate of pepsin inactivation (O-O) and EPNP incorporation (---) are plotted in both A and B. In A, the presence of Z-L-alanyl-L-tyrosine (7 X 10^-4 M) in the inactivation enzyme solution lowered the rate of inactivation (O-O) as well as the rate of incorporation (---). In B, glycyl-L-phenylalanine amide (6.82 X 10^-2 M) had a like, but more pronounced effect. (Abscissa on left represents pepsin inactivation, and on right, EPNP incorporation.)](image)

### Table IV

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Reaction time</th>
<th>Amount of EPNP protected (residues)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z-L-Alanyl-L-tyrosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>615</td>
<td>1.08</td>
</tr>
<tr>
<td>Glycyl-L-phenylalanine amide</td>
<td>135</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>615</td>
<td>1.02</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td>1.21</td>
</tr>
</tbody>
</table>

![Fig. 8. Inactivation of gastric proteases (human gastricsin, human pepsin, and bovine rennin) by epoxides. The reaction conditions were the same as described in Fig. 1.](image)

**Discussion**

EPNP apparently inactivates pepsin by forming covalent linkage to two groups of the enzyme. Since the work of Ross (21) has shown that epoxide esterifies carboxylate in an acidic solution, the esterification of 2 carboxylate residues is most likely to be the reaction responsible for inactivation. This view is supported by the alkaline lability of EPNP in EPNP-pepsin (Fig. 6), as well as by the fact that the product of this
Epoxide Inactivators of Pepsin Vol. 246, No. 14

With pepsin are probably due to (a) the fact that the inactivators...Therefore, the speed and specificity of the EPNP reaction...propylene oxide, react not only with most carboxyl groups in...shown that small epoxides, such as ethylene oxide and 1,2-...seems surprising, especially since Fraenkel-Conrat (33) has...Nevertheless, the specificity of the reaction of EPNP to pepsin...the carboxyl sites is about 5.8 (3, 13). In experiments testing...two EPNP-reactive sites, which, therefore, are likely to differ...the side chains of amino acid residues in addition to the 2 major...The two carboxyl groups modified by EPNP must differ from...Previous treatment with p-bromophenacyl bromide also results...the reaction of 2 EPNP residues in each pepsin molecule.

Therefore, epoxide-reactive sites must differ from those reactive with p-bromophenacyl bromide. This reasoning is further supported by indirect evidence: i.e., human gastricsin is completely resistant to p-bromophenacyl bromide inactivation (32), indicating the absence of the p-bromophenacyl bromide-reactive site of pepsin. However, each gastricsin molecule reacts with two EPNP-reactive sites, which, therefore, are likely to differ from the p-bromophenacyl bromide-reactive site. The reason that EPNP fails to react with diazo- and p-bromophenacyl bromide-reactive sites is obvious. In the latter case, the pK of the carboxyl sites is about 5.8 (3, 13). In experiments testing the effect of varying pH upon inactivation (pH 2.2, 3.5, and 4.6), both groups were protonated; thus, they must not react with epoxides. The data also suggest that the EPNP-sensitive groups have a pK below 2.2. The specificity of this reaction, however, cannot be explained by the low pK of these groups alone. At pH 3.5 and 4.6, many other carboxyl groups in pepsin molecules can be expected to be fully or partially ionized. Nevertheless, the specificity of the reaction of EPNP to pepsin appears to be the same at pH 4.6 as at pH 2.2. This selectivity seems surprising, especially since Fraenkel-Conrat (33) has shown that small epoxides, such as ethylene oxide and 1,2-propylene oxide, react not only with most carboxyl groups in the protein, but also with many other groups (amino, phenolic, etc.). Therefore, the speed and specificity of the EPNP reaction with pepsin are probably due to (a) the fact that the inactivators are substrate analogues, and (b) the strong nucleophilic character of the reactive carboxyl groups. The first instance is an example of affinity labeling providing specificity in enzymatic modification.

Acknowledgments—The author wishes to thank Dr. Jean Hartsuck for helpful discussions, Dr. John Mills for a sample of diazoacetyl norleucine methyl ester, and Mrs. Barbara Cox for valuable assistance in the preparation of this paper.
REFERENCES

Specific and Irreversible Inactivation of Pepsin by Substrate-like Epoxides
Jordan Tang


Access the most updated version of this article at http://www.jbc.org/content/246/14/4510

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/246/14/4510.full.html#ref-list-1