Evidence for an Essential Histidine in Carboxypeptidase Y.

REACTION WITH THE CHLOROMETHYL KETONE DERIVATIVE OF BENZYLOXYCARBONYL-L-PHENYLALANINE*

(Received for publication, December 30, 1974)

RIKIMARU HAYASHI, YASUO BAI, AND TADAO HATA
From The Research Institute for Food Science, Kyoto University, Uji, Kyoto, Japan

The possible role of histidine residues in the catalytic function of carboxypeptidase Y from bakers' yeast has been investigated using site-specific reagents. Among the reagents tested, benzyloxy-L-phenylalanylchloromethane (Z-PheCH₂Cl) was the most powerful inhibitor of the enzyme. It irreversibly inactivated both the peptidase and esterase activities with an apparent second order rate constant of 3.8 M⁻¹ s⁻¹; the D isomer caused essentially no effect on either activity. Inhibition by L-Z-PheCH₂Cl was retarded by certain competitive inhibitors of the enzyme. Using radioactive L-Z-PheCH₂Cl, the reaction with the enzyme was shown to be essentially stoichiometric. Diisopropylphosphorofluoridate (iPr₂PF)-inactivated enzyme failed to react with Z-PheCH₂Cl, and conversely, the Z-PheCH₂Cl-inhibited enzyme failed to react with radioactive iPr₂PF. Amino acid analyses of the Z-PheCH₂Cl-inactivated enzyme revealed the loss of essentially 1 residue, with a concomitant yield of a 0.62 residue of N'-carboxymethylhistidine. Since carboxypeptidase Y has a reactive serine at its active center, we concluded from these results that the mechanism involves a charge-relay system in the hydrolysis of peptide and ester substrates, as in chymotrypsin. An —SH group of carboxypeptidase Y was not affected during the reaction with L-Z-PheCH₂Cl.

The generic name “serine carboxypeptidase” has been proposed for carboxypeptidase Y and for the iPr₂PF-sensitive carboxypeptidases from plants, molds, and animal tissues, in order to distinguish them from “metal carboxypeptidases” to which carboxypeptidase A (EC 3.4.12.2) and B (EC 3.4.12.3) belong.

Carboxypeptidase Y has been obtained from bakers' yeast and characterized as an enzyme of broad specificity (2-5). Its ability to release proline is especially useful for structural studies of proteins and peptides (3, 6). The active site of this enzyme also appears to be unique. The enzyme has no essential metals (10), but has a serine hydroxyl at the active center and, thus, differs from the pancreatic carboxypeptidases A and B. The uniqueness of carboxypeptidase Y has also been shown by its strong esterase activity toward the substrate of chymotrypsin, i.e. Ac-Tyr-OEt¹ (2, 3), in contrast to the pancreatic enzymes which hydrolyze only ester substrates with a free carboxyl group in the leaving group, i.e. Bz-Gly-β-phenyllactic acid for A (7), and Bz-Gly-arginic acid for B (8). Thus, carboxypeptidase Y seems to be quite similar to chymotrypsin at its active site and in its mechanism, although the former enzyme is an exopeptidase, whereas the latter is an endopeptidase.

When we began this study, however, the following features made it difficult to define unequivocally carboxypeptidase Y as a serine enzyme. First, the enzyme displays activity at acidic pH values (4), whereas serine enzymes usually are active in the alkaline region. Second, as judged from the stoichiometric inhibition by p-HMB and chemical analyses of the enzyme (9), the enzyme has an —SH group, the functional role of which remains to be clarified. In spite of these differences, however, the high reactivity of a serine residue is common to and the most prominent feature of both carboxypeptidase Y (10) and the serine enzymes (11). Thus, it was advisable to explore the structure of the active center of carboxypeptidase Y using methods analogous to those applied to serine enzymes. In the serine enzymes, a serine residue of the active center is linked in a charge-relay system with the imidazole ring of a histidine and the carboxylate anion of an aspartic acid enhancing the nucleophilicity of the serine hydroxyl (12). Reaction with site-specific reagents toward the serine enzyme, i.e. iPr₂PF and PhCH₂SO₂F for the serine residue and chloromethyl ketone reagents such as Tos-PheCH₂Cl and Tos-LysCH₂Cl (13) for the histidine, has consistently shown the mechanism of the charge-relay system.

To explore the possible role of histidine residues in carboxypeptidase Y, its inhibition by chloromethyl ketone reagents has been studied, especially with respect to the effects on the
enzyme of the chloromethyl ketone derivative of benzoyloxy carbonyl-l-phenylalanine (Z-PheCH₂Cl). Evidence is presented showing that the inactivation by Z-PheCH₂Cl is the result of specific alkylation of a histidine residue of the enzyme.

**EXPERIMENTAL PROCEDURE**

**Materials**—Carboxypeptidase Y (Lot OY73-11) was prepared from baker's yeast ( Oriental Yeast Co.) as described previously, then lyophilized (3, 10). Z-Phe-Leu* was purchased from Fluka, and Ac-Tyr-OEt and Ac-Phe-OEt from the Protein Research Foundation. Z-D-Phe-d-Leu, Leu-Phe, and Ac-d-Phe-OEt had been previously synthesized (5). iPr₂PF and PhCH₂SO₂F were obtained from Sigma, and NaHCO₃ solutions (10–25%) were prepared by dissolving the chemical in isopropyl alcohol. [1,3,3-¹³C]iPr₂PF in propylene glycol (specific activity 0.6 mCi/mg) was purchased from New England Nuclear, and [U-¹⁴C]phenylalanine (specific activity 477 mCi/mmol) from the Radiochemical Centre. Tos-Z-PheCH₂Cl was obtained from Nakarai Chemicals. Z-PheCH₂Cl was purchased from Fox Chemicals or synthesized as described below. Z-Ala-PheCH₂Cl and Z-Ala-Gly-PheCH₂Cl were kindly supplied by Drs. K. Morihara and T. Oka of the Shionogi Research Laboratory (14). Stock solutions (0.5 mm) of the chloromethyl ketone reagents were prepared by dissolving them in methanol. A mixture of N*, N*, and N*-Cm-histidines was prepared according to Crestfield et al. (1b) and was used, without purification, to identify each component on amino acid analyses.

**Synthesis of L-Z-PheCH₂Cl, D-Z-PheCH₂Cl, and L-¹⁴CZ-PheCH₂Cl**—The l isomer of Z-PheCH₂Cl was synthesized similarly to the chloromethyl ketone of Z-Lys by the method of Coggins et al. (1b). Z-l-Phe (1 g) in tetrahydrofuran (6 ml) was converted into the mixed anhydride at -10° by the addition of triethylamine (0.9 ml) and ethyl chloroformate (0.64 ml). Then, an excess of cold ethereal diazomethane (84 ml of 0.16 M diazomethane) was added. After being maintained for 30 min at 0°, the mixture was washed with 0.1 N acetic acid and saturated aqueous NaHCO₃. The ethereal layer was dried over MgSO₄, then concentrated, and was used without purification. The d isomer of Z-PheCH₂Cl was synthesized as described above, and was crystallized from ethanol water (m.p. 101–103°). Measurement of the optical rotations of the l-Z-PheCH₂Cl and d-Z-PheCH₂Cl synthesized revealed that the reagents were true enantiomers, having equal rotations of opposite sign ([α]l -346 and +346, respectively, in 99.5% ethanol).

Z-l-[¹⁴C]Phe was synthesized from [U-¹⁴C]phenylalanine and benzoyloxy carbonyl chloride in the conventional manner, and was used for synthesis without crystallization. The radioactive phenylalanine (25 μCi) had been previously mixed with normal phenylalanine (33 mg). Immediately after treatment, the solution was successively washed with 0.1 N HCl and saturated aqueous NaHCO₃. The ether layer was dried over MgSO₄, then treated with ethanolic 4.3 M saturated aqueous NaHCO₃. The ethereal layer was dried over MgSO₄, then concentrated, and was used without purification. The d isomer of Z-PheCH₂Cl caused almost 100% inactivation of carboxypeptidase Y activity (Fig. 2).

**Environment Assays**—Peptidase activity was determined toward 0.5 mM Z-Phe-Leu at pH 6.5 by measuring the rate of decrease in the absorbance at 224 nm, as described previously (4, 9). Esterase activity was determined toward 5 mM Ac-Phe-OEt or 1 mM Ac-Tyr-OEt at pH 7.5 (4, 5) using the spectrophotometric method of Schwert and Tokenska (17). Wavelengths were 290 nm and 257 nm (17) for the hydrolysis of Ac-Phe-OEt and Ac-Tyr-OEt, respectively. Sometimes, the esterase activity against Ac-Tyr-OEt was measured at pH 8.0 by the pH-stat method (3).

**Stoichiometry and the Site of the Reaction of Z-PheCH₂Cl**—When l-[¹⁴C]Z-PheCH₂Cl was mixed with carboxypeptidase Y in 10-fold molar excess, the radioactivity incorporated increased with time of incubation and in parallel with the disappearance of enzyme activity (Fig. 4). By extrapolation to total inactivation, the radioactivity bound to the enzyme was estimated as 0.9 mol of radioactive Z-PheCH₂Cl/mol of enzyme. This value essentially accounts for a stoichiometric reaction between Z-PheCH₂Cl and the enzyme, when one corrects for the loss of activity due to denaturation during the prolonged incubation (see under "Experimental Procedure"). (The loss has tentatively been corrected as a dashed line in Fig. 3.)

**RESULTS**

**Effects of Various Chloromethyl Ketone Reagents on Carboxypeptidase Y Activity**—The effects of chloromethyl ketone derivatives of Tos-Phe, Z-Phe, Z-Ala-Phe, and Z-Ala-Gly-Phe on the peptidase and esterase activities of carboxypeptidase Y were tested as a 20-fold molar excess of reagents to protein for 8 hours of incubation. After 8 hours, Tos-PheCH₂Cl, Z-PheCH₂Cl, and Z-Ala-Gly-PheCH₂Cl showed about 15%, 50%, and 25% inactivation by both assays, respectively, while Z-Ala-PheCH₂Cl had essentially no effect on either activity. The inhibitions apparently followed first order kinetics.

The second order rate constants for inactivation were calculated by dividing the apparent first order rate constants by the inhibitor concentrations. The constants are shown in Table I and compared with those for chymotrypsin (11). Z-PheCH₂Cl was the most effective inhibitor for carboxypeptidase Y. Although the inactivation rate was somewhat slower than that with chymotrypsin, it was much faster than that with a model compound, acetylhistidine (4.5 x 10⁻³ M⁻¹ s⁻¹) (25). Replacement of the Z group by Tos resulted in a weaker inhibition of the enzyme.

**Inhibition by Z-PheCH₂Cl: Effects of Its Enantiomer, Reversible Inhibitors, and pH**—The first order rate plot for inactivation by the l isomer of Z-PheCH₂Cl is shown in Fig. 1. Here, 85% inactivation was observed after 22 hours. Parallel losses in both peptidase and esterase activities occurred. The inactivation was irreversible, as evidenced by the fact that the inactivated enzyme regained no activity when dialyzed against water. The d isomer of Z-PheCH₂Cl caused essentially no inactivation (Fig. 1).

The presence of competitive inhibitors of carboxypeptidase Y, Z-d-Phe-d-Leu, Ac-d-Phe-OEt, and trans-cinnamic acid, at concentrations equal to or greater than their respective K₅ values (5), reduced the inactivation rate by l-Z-PheCH₂Cl in both peptidase and esterase assays (Fig. 2).

The pH dependency of the second order rate constant for the inactivation by l-Z-PheCH₂Cl revealed a broad bell-shaped curve with maximum at pH 5.5 to 6.5 (Fig. 3).

**Amino Acid Analysis**—Proteins were hydrolyzed with 6 N HCl at 110° for 22 hours (21), after which amino acid analyses were performed with a Hitachi KL-5 amino acid analyzer. Half-cystine and methionine were determined as cysteic acid and methionine sulfoxide, respectively, after performic acid oxidation. The oxidation was performed at 0° for 8 hours using the method of Him (22). This method has been recommended as preventing the decomposition of histidine derivatives during oxidation (23). N'-Cm-histidine was determined using the color value of glycine derived during the same amino acid analysis (23).
TABLE I
Rate of carboxypeptidase Y and chymotrypsin inactivation by some active site-directed reagents at 25° and pH 7

| Reagents            | Carboxypeptidase Y | Chymotrypsin |
|---------------------|-------------------|--------------|
| Tos-PheCH₂Cl        | 1.6 ± 0.1         | 7.7 ± 0.3    |
| Z-PheCH₂Cl          | 3.8 ± 0.1         | 60.0 ± (11)  |
| Z-Ala-PheCH₂Cl      | 0 ± 0             | (1.4) ± 0.6  |
| Z-Ala-Gly-PheCH₂Cl  | 0.6 ± 0.0         | (100) ± 0.6  |
| iPr₄PF              | 93.3 ± 0.3        | 45 ± 0.3     |
| PhCH₃SO₃F           | 16.1 ± 0.1        | 248 ± 0.3    |

*Carboxypeptidase Y (2.3 x 10⁻⁸ M) and chloromethyl ketone reagents (4.6 x 10⁻⁵ M) were incubated at 25° in 0.09 M sodium phosphate, pH 7.0, containing 9.1% methanol. Aliquots of 20 and 50 μL were removed for the activity assay with Z-Phe-Leu and Ac-Tyr-OEt, respectively. Inactivation was followed for 8 hours with aliquots removed at 2-hour intervals. Peptidase and esterase activities were lost in parallel during incubation with the respective inhibitor. About a 5% loss of activity was observed during incubation for 22 hours in the absence of inhibitors. This loss of activity was excluded from calculations.

†Ref. 11.
*Ref. 14. Data at 40°.
°Ref. 10.

Fig. 1. Rate of carboxypeptidase Y inactivation by enantiomers of Z-PheCH₂Cl. The experimental conditions are the same as in Table I. Circles and triangles show the effect of L- and D-Z-PheCH₂Cl, respectively. ○—○ and Δ—Δ, peptidase activity; ●—● and ▲—▲, esterase activity toward Ac-Phe-OEt (spectrophotometric method).

Amino acid analyses were performed with native and L-Z-PheCH₂Cl-treated carboxypeptidase Y after performic acid oxidation. The latter enzyme lost about 90% of its activity during incubation with Z-PheCH₂Cl. Results are shown in Table II. Essentially no difference was observed with respect to the amino acid content of the two enzymes, except for the content of histidine. Carboxypeptidase Y appeared to lose 1 of the 8 histidine residues through the reaction with Z-PheCH₂Cl. This was further confirmed as follows.

Tos-PheCH₂Cl-inhibited chymotrypsin gives rise to N'-histidine upon performic acid oxidation. The yield is much less than 1 mol/mol of the enzyme, since oxidation of the ketone group may occur at either side of the α carbon atom (26). Performic acid oxidation of Z-PheCH₂Cl-inhibited carboxypeptidase Y gave 0.62 residue of N'-Cm-histidine (Table II). Neither N'' nor N'''-Cm-histidine was detected on amino acid analyses. Along with the loss of approximately 1 histidine residue, this constitutes evidence that inactivation of the enzyme by Z-PheCH₂Cl proceeds through the alkylation of a single histidine residue at the N'' position.

A methionine residue of α-chymotrypsin is alkylated by chloromethyl ketone reagents (27). However, amino acid analyses of Z-PheCH₂Cl-treated carboxypeptidase Y after performic acid oxidation revealed that the methionine content was practically unchanged, as determined from methionine sulfone (Table II). The reaction site of chloromethyl ketone reagents has also been reported to be a cysteine residue in thiol enzymes (28-31). However, the -SH group of carboxypeptidase Y was not affected during the reaction with Z-PheCH₂Cl, as judged from the Ellman reaction and from determination of S-Cm-cysteine (Table II). This was further confirmed by the constant content of cysteic acid in the native and Z-PheCH₂Cl enzymes. The tyrosine content was also not affected during the reaction with Z-PheCH₂Cl, as judged from amino acid analyses performed on samples without performic acid oxidation.

Amino acid analysis of carboxypeptidase Y from Oriental Yeast showed a slight difference from that of the enzyme from Fleischmann's yeast (3). One less histidine and 2 less methionine residues were found in the former enzyme.

Interrelationships of the Reactions of p-HMB, iPr₄PF, and Z-PheCH₂Cl with Carboxypeptidase Y Carboxypeptidase Y has a serine (9, 10), a histidine, and a cysteine (9) residue which stoichiometrically, respectively, reacted with iPr₄PF.
Fig. 4. Incorporation of L-[14C]Z-PheCH,Cl into carboxypeptidase Y. Carboxypeptidase Y (4.8 × 10⁻⁴ M) and L-[¹⁴C]Z-PheCH,Cl (4.6 × 10⁻⁶ M) were incubated in 0.09 M sodium phosphate, pH 7.0, containing 9.1% methanol at 25°C. At appropriate intervals, aliquots were removed for the assay of peptide activity (10 μl) and the determination of radioactivity (1 ml). The latter aliquot was washed three times with ether which had been previously saturated with water, then it was dialyzed against distilled water at 5°C for 30 hours. The radioactivity was determined with a 1-ml aliquot of the dialyzed solution using a Packard liquid scintillation counter (model 2002). Remaining activity was 100, 85, 66, 51, 40, 23, and 18% after incubation with Z-PheCH,Cl for 0.01, 2, 5, 9, 14, 21, and 39 hours, respectively. Dashed line was drawn by correcting the 5% and 10% losses of activity during incubation for 21 and 39 hours, respectively, in the absence of Z-PheCH,Cl.

L-Z-PheCH,Cl, and p-HMB. The interrelationships of these reactions are interesting (Table III). The stoichiometry of the reaction with radioactive iPr₂PF and L-Z-PheCH,Cl is shown in the table. The observed value of 0.74 mol of [¹⁴C]Z-PheCH,Cl bound/mole of enzyme should be corrected for the 9% remaining activity and the 10% loss by denaturation during incubation with the inhibitor, giving 0.83 mol/mole.

If Z-PheCH,Cl treatment was followed by reaction with [¹⁴C]iPr₂PF, the incorporation of radioactive iPr₂PF was prevented. (The observed value of 0.13 mol of [¹⁴C]iPr₂PF/mole of enzyme is in accord with the 9% remaining activity after pre-treatment with Z-PheCH,Cl.) Conversely, if the enzyme was first inactivated with iPr₂PF, the reaction of L-[¹⁴C]Z-PheCH,Cl was prevented. If p-HMB was added, or, if the enzyme was heat-denatured, the reaction with radioactive Z-PheCH,Cl was also largely prevented. The appreciable amounts of radioactivity incorporated into the heat-denatured enzyme may be accounted for by nonspecific reactions of [¹⁴C]Z-PheCH,Cl with various side chains of the denatured protein (15).

DISCUSSION

The reaction of Z-PheCH,Cl and Tos-PheCH,Cl toward carboxypeptidase Y is somewhat slower than that toward chymotrypsin; unlike the serine proteases (14, 16, 33-35), the chain length of the chloromethyl ketone reagents (peptide chloromethyl ketones) is not effective in enhancing the inactivation (see Table I). Nevertheless, the following evidence shows that Z-PheCH,Cl reacts with the active center of carboxypeptidase Y in an enzymatically promoted reaction. (a) The optical isomer of L-Z-PheCH,Cl has essentially no effect on enzyme activity, in accord with the stereospecificity of the enzyme (4, 5). (b) Inactivation by Z-PheCH,Cl is reduced in the presence of competitive inhibitors. (In spite of the small Kᵢ value, Leu-Phe had no effect on reducing the inactivation rate, for unknown reason(s).) (c) The pH dependence of the inactivation is similar to the pH dependence of the enzymatic hydrolysis of some peptides. (d) A single mole of [¹⁴C]Z-PheCH,Cl is incorporated into the enzyme molecule with complete loss of activity, while no incorporation occurred into the heat-denatured enzyme (13). (e) The iPr₂PF-inactivated enzyme fails to react with Z-PheCH,Cl, and, conversely, the Z-PheCH,Cl-inhibited enzyme fails to react with iPr₂PF. In addition, the reaction site of Z-PheCH,Cl was unequivocally shown to be the N' position of a histidine residue of the enzyme.

Carboxypeptidase Y (0.9 μmol) was incubated with 1.25 μmol of L-[¹⁴C]Z-PheCH,Cl in 0.09 M sodium phosphate, pH 7.0, containing 4% methanol in a total volume of 52.5 ml at 25°C. 1.5 and 1.0 μmol of normal L-Z-PheCH,Cl were successively added after 38 and 62 hours, respectively. In the final incubation stage, the methanol concentration was 14.4%. After the completion of incubation, 10% of the original activity remained. The mixture was extracted with water saturated ether, then dialyzed against distilled water at 5°C for 30 hours, and finally lyophilized. Amino acid analyses were performed after performing acid oxidation (22) and acid hydrolysis (21). Values are the averages of the analyses of three hydrolysates.

### Table II

| Residues          | Native enzyme | Z-PheCH,Cl enzyme | Fleischmann's yeast enzyme* |
|-------------------|---------------|-------------------|-----------------------------|
|                   | residues/molecule | residues/molecule | residues/molecule          |
| S-Cm-cysteine     | 1.07          | 1.07              | 1.07                        |
| —SH group (DTNB)  | 1.04          | 0.85              | 1.04                        |

*Ref. 3.

Values for cysteic acid (99), threonine (91), and serine (91) were corrected for 5%, 5%, and 10% destruction, respectively, during acid hydrolysis.

*Calculated using the glycine color value (23).

S-Cm-cysteine and the —SH group were determined without performic acid oxidation. See the text for details. DTNB, 5,5'-dithiodiopic acid (2-nitrobenzoic acid).

TABLE II

Amino acid analysis of native and Z-PheCH,Cl-treated carboxypeptidase Y after performic acid oxidation

Carboxypeptidase Y (0.9 μmol) was incubated with 1.25 μmol of L-[¹⁴C]Z-PheCH,Cl in 0.09 M sodium phosphate, pH 7.0, containing 4% methanol in a total volume of 52.5 ml at 25°C. 1.5 and 1.0 μmol of normal L-Z-PheCH,Cl were successively added after 38 and 62 hours, respectively. In the final incubation stage, the methanol concentration was 14.4%. After the completion of incubation, 10% of the original activity remained. The mixture was extracted with water saturated ether, then dialyzed against distilled water at 5°C for 30 hours, and finally lyophilized. Amino acid analyses were performed after performing acid oxidation (22) and acid hydrolysis (21). Values are the averages of the analyses of three hydrolysates.
proteases, which are easily alkylated by these reagents. The
eties are in marked contrast to those of -SH groups of thiol
exhibited no reactivity toward the -SH group. These proper-
not available to react with either iodoacetate or iodoacetamide
by a stoichiometric reaction of p-HMB (9). The -SH group is
differing features of the pH dependence probably arise from a
boxypeptidase Y. This charge-relay system in carboxypeptidase Y. This charge-relay
in the acid region; therefore, they seem to belong to the family of “acid
carboxypeptidases” originally described by Zuber and Matile
(42). The enzymes utilizing the serine protease mechanism
should not be referred to as “acid carboxypeptidases”, since
this term is closely associated with acid proteases. Acid
proteases, i.e., pepsin, renin, and a number of mold enzymes,
have recently been shown to have a common catalytic center,
aaspartic acid (55). According to the classification of proteases
by Hartley (56) (serine, thiol, metal, and acid proteases),
carboxypeptidases Y should be a serine protease, but neither
metal nor an acid protease. The enzyme also differs from thiol
proteases. Thus, carboxypeptidase Y and probably the family of enzymes
described above should more appropriately be referred to by the generic name “serine carboxypeptidases” to
distinguish them from “metal carboxypeptidases” to which
carboxypeptidases A and B belong.

Acknowledgments—Thanks are due for consultation with Dr. Takeshi Igarashi and Dr. Sho Takahashi on the synthesis of Z-PheCH,Cl and for the generous cooperation of Dr. Kazuyuki Morihara and Dr. Tatsushi Oka in providing samples of Z-Ala-PheCH,Cl and Z-Ala-Gly-PheCH,Cl. We also appreciate the counsel of Dr. Seiyo Sano throughout this study.

REFERENCES
1. Hayashi, R., Bai, Y., and Hata, T. (1974) J. Biochem. (Tokyo) 76, 1355–1357
2. Hayashi, R., and Hata, T. (1972) Biochim. Biophys. Acta 263, 673–679
3. Hayashi, R., Moore, S., and Stein, W. H. (1973) J. Biol. Chem. 248, 2596–2599
4. Hayashi, R., Bai, Y., and Hata, T. (1975) J. Biochem. (Tokyo) 77, 80–84
5. Bai, Y., Hayashi, R., and Hata, T. (1975) J. Biochem. (Tokyo) 77, 85–88
6. Hayashi, R., Moore, S., and Merrifield, R. B. (1973) J. Biol. Chem. 248, 3888–3892
7. McClure, W. O., Neurath, H., and Walsh, K. A. (1964) Biochemistry 3, 1897–1901
8. Wolf, E. C., Schirmer, E. W., and Folk, J. E. (1962) J. Biol. Chem. 237, 3094–3099
9. Hayashi, R., Moore, S., and Stein, W. H. (1973) J. Biol. Chem. 248, 8366–8369
10. Hayashi, R., Bai, Y., and Hata, T. (1975) J. Biochem. (Tokyo) 77, 1313–1318
11. Shaw, E. (1972) Methods Enzymol. 25, 655–660

*V Bai, and R Hayashi, in preparation.
12. Blow, D. M., Birktoft, J. J., and Hartley, B. S. (1969) Nature 221, 337-340
13. Shaw, E. (1967) Methods Enzymol. 11, 677-685
14. Morihara, K., and Oka, T. (1970) Arch. Biochem. Biophys. 138, 526-531
15. Crestfield, A. M., Stein, W. H., and Moore, S. (1963) J. Biol. Chem. 239, 2413-2420
16. Coggins, J. R., Kray, W., and Shaw, E. (1974) Biochem. J. 137, 579-585
17. Schwert, G. W., and Takenaka, Y. (1955) Biochim. Biophys. Acta 16, 570-575
18. Aibara, S., Hayashi, R., and Hata, T. (1971) Agr. Biol. Chem. 35, 658-666
19. Crestfield, A. M., Moore, S., and Stein, W. H. (1963) J. Biol. Chem. 238, 622-627
20. Ellman, G. L. (1959) Arch. Biochem. Biophys. 145, 70-77
21. Moore, S., and Stein, W. H. (1963) Methods Enzymol. 6, 819-831
22. Petta, P. H., Cohen, W., and Shaw, E. N. (1965) Biochim. Biophys. Res. Commun. 21, 612-618
23. Kuhn, R. W., Walsh, K. A., and Neurath, H. (1974) Biochemistry 13, 3871-3877
24. Shaw, E., and Ruscica, J. (1971) Arch. Biochem. Biophys. 145, 484-489
25. Shaw, E., and Ruscica, J. (1971) Arch. Biochem. Biophys. 145, 484-489
26. Stevenson, K. J., and Smillie, L. B. (1965) J. Mol. Biol. 12, 937-941
27. Stevenson, K. J., and Smith, L. B. (1968) Can. J. Biochem. 46, 1357-1370
28. Murachi, T., and Kato, K. (1967) J. Biochem. (Tokyo) 62, 627-629
29. Stein, M. J., and Lienor, I. E. (1967) Biochem. Biophys. Res. Commun. 26, 376-381
30. Whitaker, J. R., and Perez-Villasenor, J. (1968) Arch. Biochem. Biophys. 124, 70-76
31. Porter, W. H., Cunningham, L. W., and Mitchell, W. M. (1971) J. Biol. Chem. 246, 7675-7682
32. Moore, S., and Stein, W. H. (1964) J. Biol. Chem. 211, 905-913
33. Powers, J. C., and Tuby, P. M. (1973) Biochemistry 12, 4767-4774
34. Thomson, A., and Dennis, I. S. (1973) Eur. J. Biochem. 38, 1-5
35. Thompson, R. C., and Blout, E. R. (1973) Biochemistry 12, 44-47
36. Baker, B. R. (1967) Design of Active-Site-Directed Irreversible Enzyme Inhibitors pp. 129-149. John Wiley & Sons, New York, London, Sydney
37. Rierian, J. (1973) Biochemistry 12, 3915-3923
38. Bender, M. L., Clement, G. E., Kizdy, F. J., and Heck, H. d.-A. (1964) J. Am. Chem. Soc. 86, 3680-3689
39. Schoellmann, G., and Shaw, E. (1963) Biochemistry 2, 252-255
40. Birktoft, J. J., and Blow, D. M. (1972) J. Mol. Biol. 68, 187-240
41. Zuber, H. (1964) Nature 201, 613
42. Zuber, H., and Mattile, P. H. (1968) Z. Naturforsch. 23b, 663-665
43. Visuri, K., Mikola, J., and Enari, J.-M. (1969) Eur. J. Biochem. 7, 139-190
44. Tschesche, H., and Kupfer, S. (1972) Eur. J. Biochem. 25, 33-36
45. Carey, W. F., and Wells, J. R. E. (1972) J. Biol. Chem. 247, 5573-5579
46. Ihe, J. N., and Dure, L. S., III (1972) J. Biol. Chem. 247, 5034-5040, 5041-5047
47. Kubota, Y., Shojo, S., Funakoshi, T., and Ueki, H. (1973) J. Biochem. (Tokyo) 74, 757-779
48. Ichishima, E. (1972) Biochim. Biophys. Acta 258, 274-278
49. Jones, S. R., and Hofmann, T. (1972) Can. J. Biochem. 50, 1297-1310
50. Nakatani, T., Nasuno, S., and Iguchi, N. (1973) Agr. Biol. Chem. 37, 1237-1251
51. Logunov, A. I., and Orekhovich, V. N. (1972) Biochim. Biophys. Res. Commun. 46, 1161-1168
52. Dunn, N. W., and McQuillan, M. T. (1971) Biochim. Biophys. Acta 235, 149-158
53. Shaw, D. C., and Wells, J. R. E. (1972) Biochem. J. 128, 229-235
54. Hofmann, T., Lee, H., Jones, S. R., and Murachi, T. (1974) Fed. Am. Soc. Exp. Biol. Abstract 476
55. Truton, J. S. (1974) Accts. Chem. Res. 7, 241-246
56. Hartley, B. S. (1969) Annu. Rev. Biochem. 29, 46-72
Evidence for an essential histidine in carboxypeptidase Y. Reaction with the chloromethyl ketone derivative of benzylloxycarbonyl-L-phenylalanine.

R Hayashi, Y Bai and T Hata

J. Biol. Chem. 1975, 250:5221-5226.

Access the most updated version of this article at http://www.jbc.org/content/250/13/5221

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/250/13/5221.full.html#ref-list-1