Reaction of Yeast Carboxypeptidase C[1] with Group-Specific Reagents†

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ABSTRACT: The reactions between yeast carboxypeptidase C and the group-specific reagents, phenylglyoxal and iodoacetamide, have been studied in detail and the reactions of residue at the active site with *N*-tosyl-L-phenylalanine chloromethyl ketone and diisopropyl phosphorofluoridate have been confirmed. Modification of the enzyme by either phenylglyoxal or iodoacetamide results in the loss of peptidase activity, while esterase activity remains unchanged. Inactivation by phenylglyoxal appears to be the result of the modification of a single arginine residue, whereas inhibition by iodoacetamide can be correlated with the modification of a single methionine residue. Inactivation of the enzyme by either *N*-tosyl-L-phenylalanine chloromethyl ketone or diisopropyl phosphorofluoridate is the result of the modification of a single histidine and a single serine residue, respectively. The pattern of inhibition indicates certain analogies in the mechanism of yeast carboxypeptidase C to pancreatic chymotrypsin, on the one hand, and to carboxypeptidase A, on the other.

The existence of two distinct classes of carboxypeptidase is now well established. One class, typified by pancreatic carboxypeptidases, exhibits maximum activity at neutral or alkaline pH and requires divalent cations on the II-B transition series (Hartsuck and Lipscomb, 1971; Folk, 1971). The second class, termed "acid carboxypeptidases" (Zuber and Matile, 1968) or "serine carboxypeptidases" (Hayashi et al., 1975a), displays maximal activity in the acid pH range and is inhibited by diisopropyl phosphorofluoridate.[1]

While the neutral or alkaline carboxypeptidases are secretion products that serve extracellular functions, the serine carboxypeptidases seem to act intracellularly. In contrast to the extensively studied bovine pancreatic carboxypeptidases A and B, much less is known of the mechanism of action of the serine carboxypeptidases.

As part of our continuing interest in the mechanism of action of carboxypeptidases, we undertook an investigation of the mechanism of action of carboxypeptidase C isolated from baker's yeast. This enzyme was first described by Hata et al. (1967a) and certain of its chemical, physical, and enzymatic properties were subsequently reported by Hata and co-workers (Hata et al., 1967b; Hayashi et al., 1970, 1972, 1973a,b, 1975b,c; Aibara et al., 1971; Hayashi and Hata, 1972) and by us (Kuhn, 1973; Kuhn et al., 1974). The enzyme was found to be a carboxypeptidase of broad specificity capable of removing proline residues from the carboxyl terminus of peptides. Subsequent kinetic studies with synthetic peptides have confirmed these characteristics (Kuhn et al., 1974; Hayashi et al., 1975b; Bai et al., 1975). The enzyme possesses both peptidase and esterase activities which are inhibited by diisopropyl phosphorofluoridate (Hayashi et al., 1972, 1973b, 1975c; Kuhn et al., 1972, 1974). We have previously described the effect of a number of inhibitors upon the activity of yeast carboxypeptidase C (Kuhn et al., 1972, 1974; Kuhn, 1973). Some similar results have also been reported (Hayashi et al., 1973a,b, 1974, 1975a,c). This paper characterizes the reaction of the enzyme with four group-specific reagents, i.e., phenylglyoxal, iodoacetamide, *N*-tosyl-L-phenylalanine chloromethyl ketone, and diisopropyl phosphorofluoridate. The inclusion in a single communication of the results of the modification of yeast carboxypeptidase C by these four group specific reagents results in a much clearer understanding of the nature of the enzyme's active site and provides valuable information as to its probable mechanism of action.

Materials and Methods

Materials. Yeast carboxypeptidase C was isolated as previously described (Kuhn et al., 1974). *N*-Acetyl-L-tyrosine ethyl ester was purchased from Fox Chemical Co., and carbobenzoxy-L-phenylalanyl-L-leucine and *N*-tosyl-L-phenylalanine chloromethyl ketone were from Cyclo Chemical Corp.

DFP[2] was purchased from Pierce Chemical Co. The reagent was diluted to 1 M in water-free isopropyl alcohol and stored over molecular sieves (Matheson Coleman and Bell). [^{14}C]-DFP was obtained from New England Nuclear Corp. and [^{32}P]DFP from Amersham/Searle Corp.

Iodoacetamide was purchased from J. T. Baker Chemical Co. and [^{14}C]iodoacetamide from New England Nuclear Corp. Phenylglyoxal was obtained from K & K Laboratories, Inc.

Sephadex was purchased from Pharmacia Fine Chemicals

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[1] The enzyme was originally named yeast protease C, to differentiate it from the yeast endopeptidases A and B (Hata et al., 1967a). Later, Hayashi et al. (1973a) proposed the name carboxypeptidase Y and more recently introduced the generic name "serine carboxypeptidase" for carboxypeptidase Y and all DFP-sensitive carboxypeptidases. We prefer the term yeast carboxypeptidase C to denote its origin and to differentiate it from yeast carboxypeptidase α, which is a metal-requiring carboxypeptidase (Felix and Brouillet, 1966).

[2] The following abbreviations are used: DFP, diisopropyl phosphorofluoridate; DIP, diisopropylphosphoryl; NPGB, *p*-nitrophenyl-*p'*-guanidinobenzoate; TosPheCH$_2$Cl, *N*-tosyl-L-phenylalanine chloromethyl ketone; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Cbz, benzyloxycarbonyl.

and Dowex 50-X2 and Dowex 1-X2 from Bio-Rad Laboratories.

Enzymatic Assays. Esterase activity was measured at 25 °C in a Radiometer TTT-1 autotitrator with 0.1 N NaOH as the titrant. The substrate, *N*-acetyl-L-tyrosine ethyl ester (0.01 M), was dissolved in 0.01 M sodium phosphate–0.15 M NaCl (pH 8.0). Exopeptidase activity was determined by measuring the decrease in absorbance at 224 nm using as substrate carbobenzoxy-L-phenylalanyl-L-leucine (1 mM) dissolved in 0.1 M sodium phosphate–0.15 M NaCl (pH 6.0).

Standardization of Radioactive Reagents. The specific activity of [^{14}C]DFP was determined by reacting purified bovine trypsin (0.94 active site reactive toward NPGB[2] per protein molecule) with 2 mM [^{14}C]DFP in 0.1 M Tris-HCl (pH 7.5), containing 0.05 M $CaCl_2$ at room temperature. Less than 1% activity remained after 10 min. After 1h, the solution was acidified to pH 2.3 by the addition of formic acid and desalted on a 2.5 × 25 cm column of Sephadex G-25 equilibrated with 0.1 M formic acid. The concentration of DIP-trypsin was measured by the absorbance at 280 nm and the number of counts incorporated was determined in a scintillation counter. The specific activity of [^{14}C]DFP was found to be 14 400 cpm/μmol.

The specific activity of [^{14}C]iodoacetamide was determined by reacting cysteine (15 μmol) with 10 μmol of [^{14}C]iodoacetamide in 1 ml of 0.1 M *N*-ethylmorpholine buffer (pH 9.0), at room temperature. After 3 h, the solution was acidified by the addition of 1 ml of 12 N HCl. The mixture was then hydrolyzed in vacuo at 110 °C for 16 h. The hydrolysate was dried, dissolved in 200 μl of water, and applied to a 5-cm line on Whatman No. 3MM paper. The resulting *S*-carboxymethylcysteine was purified by descending chromatography with butanol–acetic acid–water (4:1:1). The radioactive carboxymethylcysteine was located by staining with ninhydrin and by counting sections of 1-cm strip after elution with water. The carboxymethylcysteine content was determined by amino acid analysis, and the radioactivity by counting an aliquot of the solution. This method gave a specific activity of 43 800 dpm/μmol of iodoacetamide.

Reaction with Inhibitors. (1) Phenylglyoxal. The method of Takahashi (1968) was employed. To a solution of carboxypeptidase C (1 mg/ml) in 0.1 M sodium phosphate–0.15 M NaCl (pH 7.5), an equal volume of 3% phenylglyoxal in water was added. Peptidase and esterase activities were determined at several time intervals. For incorporation studies, aliquots were removed at the appropriate times, desalted on a 1 × 10 cm column of Sephadex G-25 equilibrated with 10% acetic acid, and hydrolyzed in vacuo for 18 h at 110 °C in the presence of 6 N HCl. The loss of arginine was determined by amino acid analysis.

(2) Iodoacetate and Iodoacetamide. Solutions of enzyme (1 mg/ml) in 0.01 M sodium phosphate–0.15 M NaCl (pH 6.5) were adjusted to either 0.2 M iodoacetate or 0.2 M iodoacetamide. Samples were removed at various times and assayed for both esterase and peptidase activities.

(3) DFP. In order to determine the stoichiometry of inhibition, the enzyme (1 mg/ml) was dissolved in 0.01 M sodium phosphate–0.15 M NaCl (pH 7.0), and sufficient 1 M [^{14}C]DFP in isopropyl alcohol was added to bring the final concentration to 1 mM. After all enzymatic activity had disappeared, excessive radioactive reagent was removed by gel filtration on a 2.5 × 20 cm column of Sephadex G-25 which had been equilibrated with 10% acetic acid. Protein concentration was determined spectrophotometrically (at 280 nm) and radioactivity by counting in a scintillation counter. For the isolation of the radioactive DIP-peptide, carboxypeptidase C (220 mg) was reacted with 1 mM [^{32}P]DFP until no detectable peptidase activity remained. Excess radioactive reagent was removed by gel filtration on a column (2.5 × 20 cm) of Sephadex G-25 equilibrated with 10% acetic acid. The fractions containing protein were lyophilized and the active site peptide was isolated as described.

(4) TosPheCH_2Cl.[2] The enzyme (1 mg/ml) was incubated with 2 mM TosPheCH_2Cl in the buffers described in the text. At various times, aliquots were removed and assayed for esterase and peptidase activities.

Reaction with Cyanogen Bromide. The general procedure of Gross (1967) was used. Carboxypeptidase C (220 mg) was dissolved in 20 ml of 70% formic acid and 220 mg of cyanogen bromide was added. The solution was allowed to stand overnight at room temperature protected from light. Water (400 ml) was added and the solution lyophilized.

Alkylation of Thiol Groups with 4-Vinylpyridine. The protein was dissolved in 1.25 M Tris-HCl (pH 7.6) containing 1 mM EDTA and 6 M guanidine hydrochloride to give a final protein concentration of 25–30 mg/ml. Dithioerythritol was added to give a 20-fold molar excess relative to the cystine concentration and the mixture stirred at room temperature for 3 h. A threefold molar excess of vinylpyridine relative to dithioerythritol was added and the mixture stirred for an additional 1.5 h at room temperature. The mixture was then adjusted to pH 2.5 and excess vinylpyridine removed on a 2.5 × 25 column of Sephadex G-25 which had been equilibrated with 9% formic acid. Fractions containing protein were pooled and lyophilized.

Results

At neutral pH, phenylglyoxal reacts with guanidinium groups of proteins forming derivatives which are stable in acidic solutions (Takahashi, 1968). Treatment of yeast carboxypeptidase C (1 mg/ml) with 1.5% phenylglyoxal in 0.1 M sodium phosphate–0.15 M NaCl (pH 7.5) resulted in a loss of peptidase activity corresponding to a pseudo-first-order rate constant of 0.011 min^{-1}, whereas esterase activity was unaffected. Inclusion of 50 mM phenylpyruvate (a competitive inhibitor) in the reaction mixture afforded some protection against inactivation (pseudo-first-order rate constant of 0.0058 min^{-1}). If the modification of arginine residues were responsible for the observed loss of peptidase activity, the number of groups modified in the protected enzyme should be lower than in the unprotected one. Modification of the unprotected enzyme occurred at a faster initial rate than did that of the protected one (Figure 1). When the number of groups modified in the protected enzyme was subtracted, the slower rate of inactivation in the presence of competitive inhibitor could be related to the protection of a single arginine residue (Figure 1, dotted line).

Reaction of the enzyme (1 mg/ml) with 0.2 M iodoacetate in 0.01 M sodium phosphate–0.15 M NaCl (pH 6.5) up to 12 h resulted in no change in either esterase or peptidase activity. However, treatment with iodoacetamide under identical conditions resulted in a selective loss of peptidase activity while esterase activity remained unaffected. Inactivation followed pseudo-first-order kinetics with a rate constant of 0.00963 min^{-1}. Inclusion of phenylpyruvate (50 mM) in the incubation mixture resulted in a marked decrease in the observed rate of inactivation (pseudo-first-order rate constant of 0.00154 min^{-1}).

The stoichiometry of the inhibition was investigated using [^{14}C]iodoacetamide (Figure 2). Extrapolation to zero activity

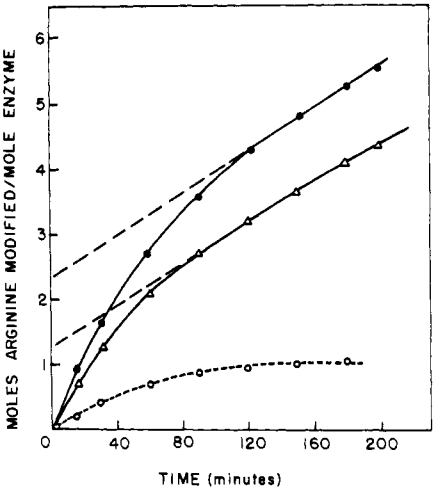

FIGURE 1: Modification of arginine residues with phenylglyoxal. Reaction conditions are described in the text. The loss of arginine with (△) or without (●) added 50 mM phenylpyruvate was determined by amino acid analysis following acid hydrolysis. The dotted line represents the difference in loss of arginine between the protected and unprotected samples.

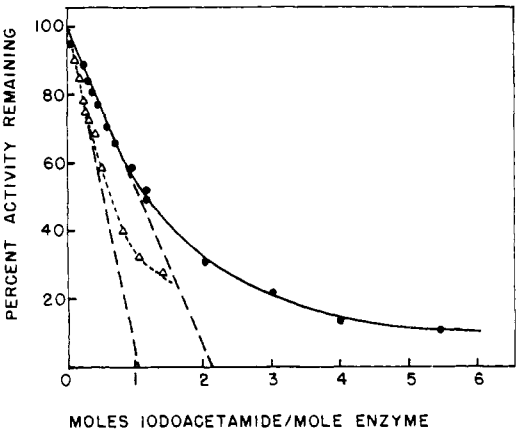

FIGURE 2: Loss of peptidase activity upon the incorporation of [^{14}C]-iodoacetamide into yeast carboxypeptidase C. The details of the experiment are given in the text. (●) Incorporation of reagent into untreated enzyme; (△) incorporation into enzyme which had been pretreated for 6 h with 0.2 M iodoacetate.

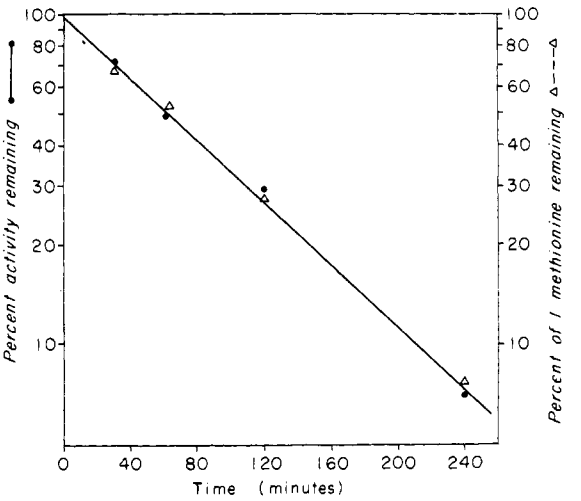

FIGURE 3: Pseudo-first-order rate of loss of percent of peptidase activity and of percent of one methionine residue upon treatment of yeast carboxypeptidase C with iodoacetamide. For details of the experiment, see the text. Solid dots represent peptidase activity; open triangles represent percentage loss of one methionine residue per molecule.

of the initially linear plot of loss of activity vs. the mole ratios of reagent/enzyme corresponded to the incorporation of two groups of iodoacetamide per enzyme molecule. If the enzyme was first treated with iodoacetate for 6 h and the incorporation of iodoacetamide then measured, the loss of peptidase activity corresponded approximately to the incorporation of one molecule of iodoacetamide per enzyme molecule. Pretreatment with iodoacetate itself had no effect on peptidase activity during the 6 h pre-incubation period. A total loss of activity was never observed upon treatment of the enzyme with iodoacetamide under the conditions of these experiments.

In order to identify the amino acid residue which upon modification caused the loss of peptidase activity, the enzyme was treated with [^{14}C]iodoacetamide until 60% of the enzyme was inactivated. Following removal of excess radioactive reagent, the enzyme was hydrolyzed in 6 N HCl and subjected to amino acid analysis, and the effluent was monitored for radioactivity. The distribution of radioactive products was identical with that observed for the acid hydrolysis of carboxymethylmethionine (Gundlach et al., 1959). Since carboxymethylmethionine is not oxidized by performic acid to the sulfone, the iodoacetamide-treated enzyme was oxidized with performic acid, hydrolyzed, and then subjected to amino acid analysis. A control sample containing enzyme but no iodoacetamide was similarly treated. No change in the amount of any amino acid was detected in the control sample, whereas the experimental sample showed a decrease only in methionine sulfone with increasing time of reaction with iodoacetamide.

As shown in Figure 3, the loss of one methionine residue correlated with loss of activity when both are plotted according to first order kinetics.

Methionine residues can be regenerated from their sulfonium salts (Naider and Bohak, 1972; Naider et al., 1972). If the modification of a methionine residue was indeed responsible for the loss of peptidase activity, activity should be regained upon treatment of the modified protein with 2-mercaptoethanol or with mercaptoacetic acid. Treatment of modified enzyme (5% residual activity) with 0.1 M 2-mercaptoethanol in 0.1 M sodium phosphate (pH 7.5) resulted in the regeneration of 52% of the original activity while treatment with 0.1 mercaptoacetic acid in the same buffer resulted in the regeneration of 32% of the original activity. Hence, both the loss of methionine upon inactivation of the enzyme by iodoacetamide and the (partial) reactivation of the inhibited enzyme by mercaptides support the conclusion that a single methionine residue was modified.

N-Tosyl-L-phenylalanine chloromethyl ketone has been shown to alkylate a specific histidine in the active site of chymotrypsin (Schoellman and Shaw, 1963). Since carboxypeptidase C hydrolyzes the chymotrypsin substrate, *N*-acetyl-L-tyrosine ethyl ester, and is also inhibited by DFP, it was of interest to probe for the involvement of a histidine residue in the esterase activity of the yeast enzyme. When the enzyme (1 mg/ml) was treated with 2 mM TosPheCH$_2$Cl, both esterase and peptidase activities were slowly lost. The pH dependence of the pseudo-first-order rate constant for inhibition in the presence of saturating amounts of TosPheCH$_2$Cl resembled that observed for ester hydrolysis (Kuhn et al., 1974), exhibiting a maximum at pH 7.4.

In order to determine the stoichiometry of the reaction, the protease (1 mg/ml) was reacted with 2 mM TosPheCH$_2$Cl in 0.1 M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic

acid–0.15 M NaCl (pH 7.0). At the appropriate levels of inhibition, samples were taken, excess reagent was removed on a column (1 × 10 cm) of Sephadex G-25, and the modified protein was oxidized with performic acid. Following acid hydrolysis, the number of residues of 3-carboxymethylhistidine per molecule of carboxypeptidase C was determined. Amino acid analysis revealed that histidine was the only residue modified. Loss of activity was proportional to the formation of 3-carboxymethylhistidine and was complete when 1.0 residue of this derivative per molecule of enzyme was formed. That an intact active site was required for reaction with the reagent was demonstrated by the similarities between the pH profile of ester hydrolysis and of inhibition by the reagent, and by the failure of the reagent to be incorporated when the enzyme was first reacted with DFP. Hayashi et al. (1974, 1975a) have independently reported similar results using $CbzPheCH_2Cl$.

Yeast carboxypeptidase C was rapidly inactivated by DFP or by phenylmethanesulfonyl fluoride. An enzyme solution of 1 mg/ml lost 80% of the initial activity in less than 0.5 min after the addition of 1 mM DFP or phenylmethanesulfonyl fluoride. To determine the stoichiometry of the reaction, the protease (1 mg/ml) was reacted with 1 mM [^{14}C]DFP of known specific activity (see Materials and Methods) in 0.01 M sodium phosphate–0.15 M NaCl (pH 7.0). Following total inactivation (about 1 min), excess radioactive reagent was removed on a 10-ml column of Sephadex G-25 which had been equilibrated with 10% acetic acid. Inactivation of the enzyme occurred concurrently with the incorporation of 0.98 mmol of DFP per 61 000 mg of protein. The stoichiometry of inactivation is in excellent agreement with that previously reported (Hayashi and Hata, 1972; Kuhn et al., 1972; Hayashi et al., 1973b).

Isolation and Structure of DIP-Peptide. In order to determine the nature of the amino acid residues surrounding the active serine residue and the possible evolutionary relationship of this peptide to those from other serine enzymes, a DIP-seryl peptide was isolated. The enzyme (220 mg) was inactivated with [^{32}P]DFP in 0.1 M sodium phosphate–0.15 M NaCl (pH 6.0). After inhibition was complete (3 h), excess radioactive reagent was removed on a Sephadex G-25 column (2.5 × 50 cm) equilibrated with 0.1 M *N*-ethylmorpholine acetate (pH 7.0). Fractions containing protein were pooled and lyophilized. The protein was treated with CNBr as described in Materials and Methods and the cyanogen bromide fragments were separated on a Sephadex G-150 column (2.5 × 100 cm) equilibrated with 18% formic acid containing 6 M urea. Fractions containing the major radioactive peak were pooled, desalted on a column of Sephadex G-25 (4 × 30 cm), equilibrated with 9% formic acid, and lyophilized. Cystine residues were reduced with dithioerythritol and alkylated with vinylpyridine as described in Materials and Methods. The reduced and pyridylethylated CNBr fragments were separated on a column of Sephadex G-150 (2.5 × 100 cm) as before. The major radioactive fraction was desalted, lyophilized, and then digested with $TosPheCH_2Cl$-treated trypsin (1% w/w) at 37 °C, pH 8.0. After base uptake had ceased (30 min), a second addition of trypsin was made and the digestion continued for one additional hour. The pH was then lowered to 2.4 with 1 N HCl and the material applied to a column of Sephadex G-50 (2.5 × 100 cm) equilibrated with 9% formic acid. Radioactive fractions were pooled and lyophilized. The tryptic peptides were further digested with chymotrypsin (1% w/w) at 37 °C, pH 7.5. After base uptake ceased (30 min), a second addition of chymotrypsin was made and the digestion continued for one additional hour. The pH was then lowered to 2.4 with 1 N HCl.

The acidified solution was applied to a 2.5 × 25 cm column of Dowex 50-X2 (200–400 mesh) equilibrated at 55 °C with 0.05 M pyridine acetate (pH 2.4). The column was eluted with the equilibrating buffer at a flow rate of 36 ml/h until ten fractions (3.6 ml each) had been collected. At this point the column was developed with a double linear gradient composed of 180 ml each of 0.05 M pyridine acetate (pH 2.4) and 0.5 M pyridine acetate (pH 3.75), followed by 180 ml each of 0.5 M pyridine acetate (pH 3.75) and 2.0 M pyridine acetate (pH 5.0) (Bradshaw et al., 1969). The effluent was collected in 3.6-ml fractions which were monitored with a Technicon autoanalyzer equipped for automatic alkaline hydrolysis and ninhydrin analysis (Hill and Delaney, 1967). Three major radioactive fractions were obtained. The major fraction was further purified on a column (0.9 × 50 cm) of Dowex 1-X2. The column was developed by applying a linear gradient composed of 95 ml each of 3% pyridine (pH 7.5), 0.5 M pyridine acetate (pH 6.0), 1 M pyridine acetate (pH 5.5), and 2.0 M pyridine acetate (pH 5.0). The column was eluted at a rate of 20 ml per h and 2.0-ml fractions were collected. The separation was monitored as described for the Dowex 50-X2 column. A single radioactive peptide, well separated from the other peptides, was isolated.

The sequence of amino acids in the isolated DIP-peptide was determined by sequential degradation using the subtractive Edman procedure described by Konigsberg (1967). The sequence of this hexapeptide was found to be:

$$H_2N\text{-Ile-Ala-Gly-Glu-Ser-Tyr-COOH}$$

The acid nature of the glutamic acid residue was demonstrated by the electrophoretic mobility of the peptide on paper and by the digestion of an aliquot of the peptide with yeast carboxypeptidase C. Measurements of radioactivity revealed that the phosphate moiety was covalently attached to the serine. This sequence is in complete agreement with the larger peptide independently obtained by Hayashi et al. (1973b).

Discussion

The studies described in this paper demonstrate that modification of any one of four different amino acid residues of yeast carboxypeptidase C (serine, histidine, methionine, and arginine) can abolish the peptidase activity. In one of these modifications (methionine), inactivation was incomplete and, in two of the modifications (arginine and methionine), the esterase activity remains unchanged. The group-specific reagents employed were: for serine, diisopropyl phosphorofluoridate or phenylmethanesulfonyl fluoride; for histidine, *N*-tosyl-L-phenylalanine chloromethyl ketone; for methionine, iodoacetamide; and for arginine, phenylglyoxal.

Treatment of the enzyme with iodoacetamide or phenylglyoxal impaired peptidase activity while esterase activity remained unchanged, suggesting that the methionine and arginine residues are located in a region of the active site which is involved in the binding of peptide but not ester substrates. However, ester and peptide hydrolysis probably involves a common mechanism since modification of either the serine or the histidine residues results in the loss of both esterase and peptidase activities.

In its susceptibility to group-specific reagents, yeast carboxypeptidase C appears to share features with both carboxypeptidase A and chymotrypsin. The resemblance to carboxypeptidase A is based on substrate specificity and on the sensitivity to arginine modification. In carboxypeptidase A the

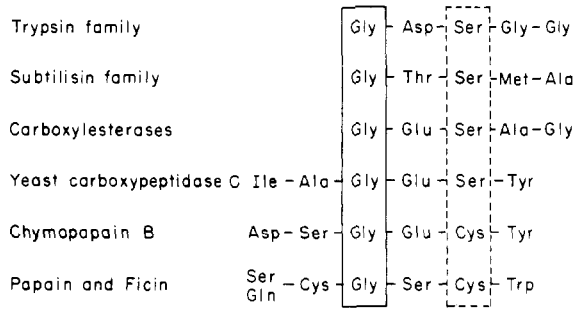

FIGURE 4: Comparison of the active site peptide from yeast carboxypeptidase C with those from other proteases. Results are discussed in the text. The residues enclosed within the solid rectangle represent features common to all of the enzymes, while those within the dotted rectangle are those directly involved in catalysis. Sequences are from Dayhoff (1972).

arginine residue is believed to bind the α-carboxyl group of peptide substrates (Lipscomb et al., 1969), and a similar role can be suggested for an arginyl residue in yeast carboxypeptidase C. The resemblance to chymotrypsin is more convincing, being based on analogous sensitivity to modification of seryl, histidyl, or methionyl residues. Thus, it is reasonable to propose that the mechanism of catalysis by yeast carboxypeptidase C is similar to that proposed for chymotrypsin where the histidine residue acts as a general base promoting a nucleophilic attack of the serine hydroxyl on the carbonyl carbon atom of the substrate (Hess, 1971). Recent evidence for the formation of an acyl-enzyme intermediate in the action of yeast carboxypeptidase C (Nakayawa and Kaiser, 1974) lends further support to the concept that the mechanism of action of this enzyme is similar to that of other serine proteases. Interpretation of the common sensitivity to methionine modification is less certain. Alkylation of Met-192 in chymotrypsin occludes the binding pocket (Koshland et al., 1962; Lawson and Schramm, 1965; Blow, 1971); similar modification of the methionine residue in yeast carboxypeptidase C may interfere with the binding of peptide substrates.

The peptide sequence around the active serine reported in this paper is consistent with that of a somewhat longer sequence independently determined by Hayashi et al. (1973b). This sequence is compared in Figure 4 with that of other hydrolases containing serine or cysteine at the active site. They share one common feature, namely a glycine residue two positions to the left of the active serine (or cysteine), but no other compelling similarity. The sequence most similar to that of yeast carboxypeptidase C is that of chymopapain B. Clearly more extensive sequence knowledge is required before any meaningful determination of the ancestral relationship between the yeast enzyme and other hydrolases can be made.

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