By-Product Analogues for Bovine Carboxypeptidase B†

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ABSTRACT: A series of monocarboxylic and dicarboxylic acid sulfur-containing by-product analogues of lysine and arginine has been synthesized and tested as competitive inhibitors of bovine carboxypeptidase B. The most effective derivatives were guanidinoethylmercaptosuccinic acid and aminopropylmercaptosuccinic acid with K_is of 4 and 8×10^{-6} M, respectively. Kinetic studies established the pure competitive nature of the inhibition. Mixed studies with the alkylating reagents bromoacetyl-D-arginine and bromoacetamidobutylguanidine established their efficiency in protecting the active-center glutamic acid and tyrosine of bovine carboxypeptidase B, respectively, from irreversible alkylation. Kinetic studies with bovine carboxypeptidase A and porcine carboxypeptidase B showed a lack of efficiency for A and high degree of efficiency for B.

Benzylsuccinic acid (BzlSA)[1] has been shown to be a very potent competitive inhibitor of bovine carboxypeptidase A (Byers and Wolfenden, 1973) and of porcine carboxypeptidase B (Zisapel and Sokolovsky, 1974). This inhibitor has been termed a "by-product analogue" that structurally resembles the products of hydrolysis of C-terminal phenylalanine peptides (Byers and Wolfenden, 1973).

We have synthesized simple sulfur-containing derivatives of arginine and lysine which are analogous in design to BzlSA and are very potent competitive inhibitors of bovine carboxypeptidase B. These compounds are GEMSA (Ia) and APMSA (IIa) (see Figure 1). The importance of the dicarboxylic acid function of these inhibitors has been demonstrated by a comparison with a series of related compounds. A preliminary account of some of these studies was reported previously (McKay and Plummer, 1977).

Materials and Methods

D,L-BzlSA (Burdick and Jackson), guanidinovaleric acid, guanidinocaproic acid (Vega-Fox), ε-aminocaproic acid (Calbiochem), MSA (Aldrich), MPA (Aldrich), MAA (Aldrich), ethylenimine (Pierce), bromopropylamine (Aldrich), and succinic acid (Eastman) were used as supplied.

Carboxypeptidases. Bovine carboxypeptidase B was obtained from pancreatic juice (Plummer, 1969). Bovine carboxypeptidase A was prepared by the method of Cox et al. (1964). Porcine carboxypeptidase B was obtained from Worthington Biochemical Corp. and was purified by affinity chromatography (Plummer, 1971). Protein concentrations were obtained assuming absorbance indices of $E_{1cm}^{1\%}$ (280 nm) as follows: bovine carboxypeptidase B, 21 (Cox et al., 1962); bovine carboxypeptidase A, 18.8 (Cox et al., 1964); and porcine carboxypeptidase B, 21.4 (Folk et al., 1960).

Enzyme and Inhibitor Assays. Enzymatic activity was determined spectrophotometrically at 254 nm (Wolff et al., 1962, as modified by Kycia et al., 1968) with hippuryl-L-arginine (Protein Research Laboratories) or hippuryl-L-argininic acid (Cyclo Chemical Co.) as substrate. Assays were carried out at 25 °C in 0.05 M Tris-HCl buffer, pH 7.6, containing 0.5 M NaCl. The inhibitor binding constants were determined graphically on Dixon plots (Dixon, 1953) using three concentrations of substrate (0.25, 0.5, and 1.0 mM for hippuryl-L-arginine; 0.125, 0.25, and 0.5 mM for hippuryl-L-argininic acid) and six concentrations of inhibitor in duplicate. Linear representation of data was determined by regression analysis. The final concentrations of bovine carboxypeptidase B were 6.15×10^{-8} M for hippuryl-L-arginine assays and 8.66×10^{-9} M for hippuryl-L-argininic acid assays.

The effect of competitive inhibitors on irreversible loss of activity of bovine carboxypeptidase B was studied using the active-site-directed alkylation reagents bromoacetyl-D-arginine (Kimmel and Plummer, 1972) and bromoacetamidobutylguanidine (Plummer, 1969). Enzyme (1.25 mg/mL) was incubated at 25 °C in 0.10 M Tris-HCl containing 0.25 M NaCl for 24 h with either a 40-fold molar excess of bromoacetyl-D-arginine at pH 7.0 or a 40-fold molar excess of bromoacetamidobutylguanidine at pH 9.0, with appropriate molar excesses of GEMSA and APMSA. At desired time intervals, 25-μL aliquots were removed and diluted 21-fold, and 25 μL of each dilution was assayed for residual activity with hippuryl-L-arginine and with hippuryl-L-argininic acid.

The effect of GEMSA on the alkylation of bovine carboxypeptidase B was also studied using [1-^{14}C]bromoacetyl-D-arginine and [1-^{14}C]bromoacetamidobutylguanidine. Reaction mixtures with 1-^{14}C-labeled alkylation reagents were prepared as outlined above but with different molar amounts and reaction times. The conditions were: bovine carboxypeptidase B (4 mg/mL), [1-^{14}C]bromoacetyl-D-arginine (40-fold molar excess), GEMSA (20-fold molar excess), and reaction time 8 h; bovine carboxypeptidase B (4 mg/mL), [1-^{14}C]-bromoacetamidobutylguanidine (40-fold molar excess), GEMSA (20-fold molar excess), and reaction time 4.5 h. Reaction mixtures of 1.8 mL were separated from excess reagents on 0.9 × 42-cm columns of Sephadex G-25, equilibrated in 0.1 M NH_4HCO_3. Protein was detected by optical density at 280 nm, and radioactivity was detected on suitable aliquots by scintillation analysis. Incorporation of label was quantitated by dividing cpm per μmol of protein by cpm per μmol of alkylating reagent.

Appropriate amounts of protein were lyophilized into hydrolysis tubes and hydrolyzed for 24 h in 6 N HCl. Radioactive

† From the Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201. *Received June 6, 1977.* This work was supported by National Institutes of Health Research Grant GM-11764, awarded by the National Institute of General Medical Sciences, PHS/DHEW.

[1] Abbreviations used are: AE-, aminoethyl-; AP-, aminopropyl-; BzlSA, benzylsuccinic acid; GE-, guanidinoethyl-; GP-, guanidinopropyl-; MAA, mercaptoacetic acid; MPA, mercaptopropionic acid; MSA, mercaptosuccinic acid; TLC, thin-layer chromatography.

0006-2960/78/0417-0401$01.00/0

TABLE I: Melting Points and Elemental Analysis of Competitive Inhibitors of Bovine Carboxypeptidase B.

Compound	Mp (°C) (not corr)	Formula	% calcd/% determined[a] C	H	N	S
GEMSA (Ia)	186–189	$C_7H_{13}N_3O_4S$	35.74	5.57	17.86	13.63
			35.84	5.51	17.95	13.24
GEMPA (Ib)	235–240	$C_6H_{13}N_3O_2S$	37.68	6.85	21.97	16.77
			37.76	6.76	21.93	16.77
GEMAA (Ic)	245–247	$C_5H_{11}N_3O_2S$	33.89	6.26	23.71	18.09
			33.96	6.15	24.03	17.39
APMSA (IIa)	189–190	$C_7H_{13}NO_4S$	40.57	6.32	6.76	15.47
			40.48	6.31	6.70	15.41
APMPA (IIb)	162–164	$C_6H_{13}NO_2S$	44.15	8.03	8.58	19.64
			43.85	8.18	8.44	18.97
APMAA (IIc)	176–179	$C_6H_{12}NO_4SNa$[b]	33.17	5.57	6.45	14.76
			33.32	5.47	6.49	13.75
AEMSA (III)	80–85	$C_6H_{11}NO_4S$	37.30	5.74	7.25	16.59
			37.22	5.85	7.39	16.47
GPMSA (IV)	93–96	$C_8H_{15}N_3O_4S$	38.54	6.07	16.86	12.86
			38.82	6.28	16.71	12.68

[a] Analyses were performed by Instranal Laboratory, Inc., Rensselaer, New York. [b] Sodium formate salt.

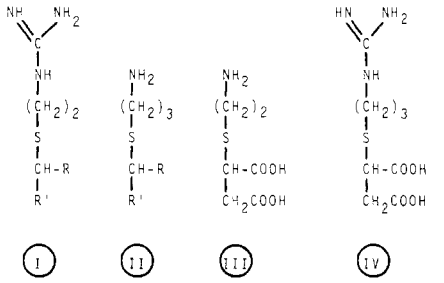

FIGURE 1: Structures of competitive inhibitors. Substitutions: (a) R = COOH, R′ = CH_2COOH; (b) R = H, R′ = CH_2COOH; and (c) R = COOH, R′ = H.

products were identified on an amino acid analyzer by monitoring column effluents with a 2-mL flow cell (Nuclear-Chicago, Model 6782) coupled to a liquid radiochromatography system (Nuclear-Chicago, Model 4526) and a recorder. The areas of radioactivity were weighed to determine the amount of each alkylated amino acid derivative.

Synthetic Procedures. Radioactive active-site-directed alkylation reagents were synthesized from [1-^{14}C]bromoacetic acid (New England Nuclear) by the following method.

Isobutyl chloroformate (9.78 mmol) was added to 22 mL of chilled tetrahydrofuran (−12 °C) containing bromoacetic acid (1 mCi of radioactive material plus nonradioactive diluent, 9.06 mmol) and *N*-methylmorpholine (10.11 mmol). The slurry was swirled by hand in a −12 °C water bath for 5 min, and 22 mL of a chilled aqueous solution (4 °C) containing D-arginine-HBr or agmatine-HBr (9.06 mmol) and *N*-methylmorpholine (9.15 mmol) was added. The mixture was allowed to warm to room temperature with gentle stirring. After 50 min the pH was lowered to 4.3 with 3 mL of glacial acetic acid, and most of the tetrahydrofuran was removed by gentle flash evaporation (10 min, 32 °C water bath). The remaining solution was cooled to 4 °C and divided into two parts. Each part was applied to a 2.5 × 180 cm column of Sephadex G-10 equilibrated at 4 °C in 0.1 N acetic acid at a flow rate of 23.6 mL cm^{-2} h^{-1}. Effluent fractions of 10 mL were collected. Product was detected by ninhydrin reaction of suitable aliquots (Moore, 1968) followed by lyophilization of the most retarded peak (Plummer, 1971). The yield was 55% for the arginine derivative and 65% for the agmatine derivative.

By-product analogues were prepared from appropriate mercaptocarboxylic acids by reaction with either ethylenimine (Raftery and Cole, 1966) or bromopropylamine. The amino compounds were guanidinated with 2-guanyl-3,5-dimethylpyrazole (Bannard et al., 1958). All compounds were purified by column chromatography and characterized by elemental analysis for C, H, N, and S (Table I). Amino derivatives were chromatographed on an amino acid analyzer, and each was verified as a single peak. The following procedures are representative.

(a) *AEMSA (III).* Mercaptosuccinic acid (53.2 mmol) was dissolved in 1 M $NaHCO_3$, pH 9.0 (200 mL), and the pH was readjusted to 9.0 with 5 N NaOH. Ethylenimine (193.2 mmol) was added, and the pH rose to 9.4. The extent of reaction was monitored by following the disappearance of free sulfhydryl groups by the method of Ellman (1959). The reaction was complete in 30 min. The pH was lowered to 1.7 with concentrated HCl, and the mixture was applied to a column of Dowex 50-X8 (Aminex Q150S, Bio-Rad), 2.5 × 42 cm, in the H^+ form. The column was washed with 0.1 N HCl for 4 h at a flow rate of 39.6 mL cm^{-2} h^{-1}. Elution was initiated at 50 °C with 0.2 M pyridine formate, pH 3.26. Fractions of 25 mL were collected. Product was detected by ninhydrin reaction of suitable aliquots (Moore, 1968). Pertinent fractions were pooled and lyophilized; this material was suitable for use in the subsequent guanidination reaction.

An analytical sample, which was also used for kinetic studies, was prepared by dissolving a portion of the above product (500 mg) in 2 N HCl (10 mL) and applying it to the Dowex column equilibrated in 0.2 M pyridine formate, pH 2.3. After 3 h the equilibration buffer was automatically replaced with a constant-volume elution reservoir (500-mL capacity) that allowed a gradient from the initial buffer to 0.4 M pyridine formate, pH 5.28. Fractions were collected, analyzed, pooled, and lyophilized as before. These chromatographic conditions were suitable for all amino derivatives.

(b) *GEMSA (Ia).* AEMSA (49.7 mmol) was dissolved in water (100 mL), and the first of two aliquots of 2-guanyl-3,5-dimethylpyrazole nitrate (49.7 mmol) was added. The pH was adjusted to 9.5 with 5 N NaOH, and the reaction was stirred overnight under a N_2 atmosphere. Some free amino groups were still present, as detected by TLC strips sprayed with ninhydrin. The second aliquot was then added, the pH was readjusted to 9.5, and the reaction was stirred overnight again. The pH of the reaction was lowered to 1.0 with concentrated

TABLE II: Inhibitors of Bovine Carboxypeptidase B.[a]

Inhibitor	K_i (mM)
Dicarboxylic acids	
D,L-GEMSA	0.004[b]
D,L-APMSA	0.008[b]
D,L-BzlSA	0.035[b]
D,L-GPMSA	0.015
D,L-AEMSA	0.075
Succinic acid[c]	>25
Monocarboxylic acids	
GEMPA	1.5
Guanidinocaproic acid	1.7
GEMAA	1.1
Guanidinovaleric acid[d]	0.6
APMPA	3.6
APMAA	1.4
ε-Aminocaproic acid[d]	0.7

[a] Substrate hippuryl-L-arginine. [b] Similar K_i, substrate hippuryl-L-argininic acid. [c] pH readjusted to 7.6 with NaOH. [d] Reported by Folk and Gladner (1958) to be a competitive inhibitor of bovine carboxypeptidase B.

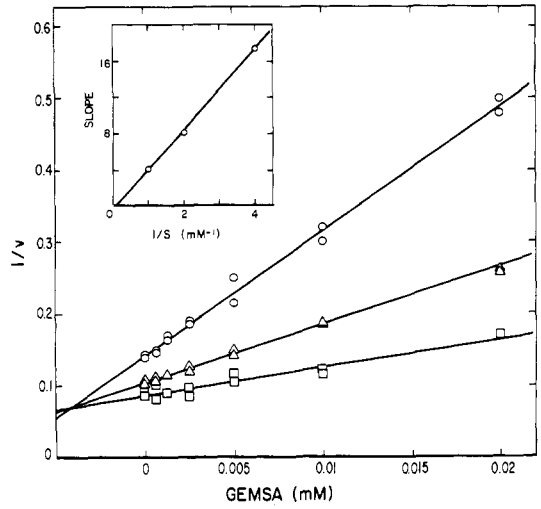

FIGURE 2: Dixon plot of the rate of hydrolysis of hippuryl-L-arginine (in 0.05 M Tris-HCl, pH 7.6, 0.5 M in NaCl at 25 °C) by bovine carboxypeptidase B (at 6.15×10^{-8} M) as a function of concentration of GEMSA. Substrate concentrations are 1 mM (□), 0.5 mM (△), and 0.25 mM (○). Inset: Replot of slope as a function of inverse substrate concentration ($1/S$).

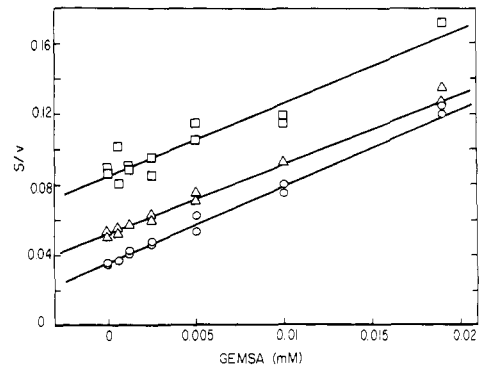

FIGURE 3: Cornish-Bowden plot of the hydrolysis of hippuryl-L-arginine by bovine carboxypeptidase B as a function of GEMSA concentration at substrate concentrations of 1 mM (□), 0.5 mM (△), and 0.25 mM (○). Conditions are as in Figure 2.

HCl, and the mixture was applied to a 2.5 × 42 cm column of Dowex 50-X8 in the H^+ form. The column was washed with 0.1 N HCl for 4 h as above and eluted with 0.4 M pyridine formate, pH 5.26. Fractions of 25 mL were collected. Guanidino compounds were detected in suitable aliquots by Sakaguchi analysis (modification of Tomlinson and Viswanatha, 1974). Pertinent fractions were pooled and lyophilized. Analytically pure material was obtained by rechromatographing the pooled fractions on the Dowex column equilibrated with 0.2 M pyridine formate, pH 3.26, followed by a gradient to 0.4 M pyridine (500-mL constant-volume elution reservoir) using the aforementioned conditions. These conditions were suitable for all guanidino compounds. The overall yield of GEMSA was 41% based on MSA.

(c) *APMSA (IIa).* Mercaptosuccinic acid (26.6 mmol) was dissolved in water (100 mL), and the pH was adjusted to 8.5 with 9.5 NaOH. Bromopropylamine (26.6 mmol) was dissolved in water (50 mL), and the pH was adjusted to 8.0 with NaOH. The two solutions were mixed, and the pH was maintained at 8.1 with 9.5 N NaOH by an autotitrator. After 19 h the pH was lowered to 1.3 with concentrated HCl and the mixture was applied to a 2.5 × 42 cm column of Dowex 50-X8. Chromatographic conditions and subsequent rechromatography with a gradient for an analytical sample were identical to those described for AEMSA. The yield of APMSA was 90% based on MSA.

Results

Competitive Inhibitors. The inhibitor binding constants (K_i) for our various derivatives are listed in Table II. The Dixon plots (Figure 2) were typical of competitive-type inhibition of bovine carboxypeptidase B. Competitive kinetics were confirmed by replots of slope vs. $1/S$ (Figure 2, insert), which gave straight lines intersecting at the origin, and by Cornish-Bowden plots (Cornish-Bowden, 1974) (Figure 3), which gave parallel lines. GEMSA (Ia) and APMSA (IIa), the sulfur-containing dicarboxylic acid analogues of arginine and lysine, respectively, are the best competitive inhibitors of bovine carboxypeptidase B and are somewhat more tightly bound than BzlSA. GPMSA (IV) and AEMSA (III) are similar derivatives, having one methylene more and less than arginine and lysine, respectively, and a less efficient K_i. No monocarboxylic acid was a very effective inhibitor, the best being approximately 150-fold less active than GEMSA.

Effect of Competitive Inhibitors on Active-Center Alkylation. The results of competition experiments between the dicarboxylic acid analogs GEMSA and APMSA and active-center-directed, irreversible inhibitors are shown in Table III. Both compounds markedly protect bovine carboxypeptidase B against alkylation by bromoacetyl-D-arginine and by bromoacetamidobutylguanidine. The compounds were particularly effective at pH 7.0 against bromoacetyl-D-arginine. GEMSA at a concentration less than equimolar to enzyme (0.4) and APMSA at an equimolar concentration increased the time for 50% inhibition from 3.1 to 7.4 and 10.8 h, respectively. GEMSA and APMSA, when incubated with enzyme alone at concentrations of 26-fold over enzyme, showed no inhibition after dilution and no irreversible loss of activity after 24 h at either pH 7.0 or 9.0.

The protective effects of GEMSA against alkylation at the active center were reaffirmed by studies with radioactive inhibitors (Table IV). GEMSA at a 20-fold molar excess protected carboxypeptidase B from alkylation at the active-center glutamic acid by a 40-fold molar excess of [1-^{14}C]bromoacetyl-D-arginine. Incorporation of radioactivity was 0.95 residue

TABLE III: Effect of Competitive Inhibitors on Irreversible Loss of Activity of Bovine Carboxypeptidase B Incubated with Bromoacetyl-D-arginine or Bromoacetamidobutylguanidine.

Alkylating reagent	Competitive inhibitor	Molar ratio (alkylating reagent/inhibitor)[a]	Time for 50% inhibition (h)
Bromoacetyl-D-arginine[b]			
	None		3.1
	GEMSA	40:0.4	7.4
		40:2.0	50.0[c]
	APMSA	40:1.0	10.8
		40:4.0	70.0[c]
Bromoacetamidobutyl guanidine[d]			
	None		3.0
	GEMSA	40:6.7	10.9
		40:26.7	50.0[c]
	APMSA	40:6.7	7.0
		40:26.7	14.0

[a] Molar excess of inhibitors over enzyme. [b] pH 7.0. Alkylates active-center glutamic acid 270 (Kimmel and Plummer, 1972). [c] Values extrapolated from shorter time periods. [d] pH 9.0. Alkylates active-center tyrosine-248 (Plummer, 1969).

TABLE IV: Radioactive Amino Acid Derivatives of Bovine Carboxypeptidase B after Incubation with [1-^{14}C]Bromoacetyl Affinity Alkylating Reagents.

Radioactive product[a]	Derivatives present (μmol/μmol of enzyme) after reaction[b] with [1-^{14}C]Bromoacetyl-D-arginine		[1-^{14}C]Bromoacetamidobutylguanidine	
	Alone	Plus GEMSA	Alone	Plus GEMSA
Acid unstable[c]	0.99	0.01	0.14	0.04
ε-Dicarboxymethyllysine	0.0	0.0	0.18	0.14
1-Carboxymethylhistidine	0.02	0.03	0.07	0.05
3-Carboxymethylhistidine	0.04	0.06	0.22	0.18
O-Carboxymethyltyrosine	0.0	0.0	0.86	0.30
ε-Carboxymethyllysine	0.0	0.0	0.64	0.53
Total radioact. incorp.	1.05	0.10	2.14	1.27
% act. remaining	5	100	19	75

[a] Radioactive derivatives of amino acids were detected and quantitated as outlined under Materials and Methods. After acid hydrolysis, essentially all of the radioactivity was recovered in known compounds. [b] Reaction conditions: Carboxypeptidase B, 4 mg/mL; GEMSA, 20-fold molar excess; [1-^{14}C]bromoacetyl-D-arginine, 40-fold molar excess at pH 7.0; [1-^{14}C]bromoacetamidobutylguanidine, 40-fold molar excess at pH 9.0. [c] The ester formed by alkylation of the active-site glutamic acid is unstable to acid hydrolysis. The resultant radioactive glycolic acid is eluted from the amino acid analyzer as a nonretarded peak.

TABLE V: Inhibition of Bovine Carboxypeptidase A and Porcine Carboxypeptidase B by Dicarboxylic Acid Inhibitors.

Inhibitor	Bovine carboxypeptidase A K_i (μM)[a]	Porcine carboxypeptidase B K_i (μM)[b]
D,L-BzlSA	1.1[c]	1.2[d]
	0.4	8
D,L-GEMSA	>5000[e]	1
D,L-APMSA	>5000[e]	5

[a] Substrate hippuryl-L-phenylalanine at 0.22 and 0.44 mM; enzyme at 8.6×10^{-8} M. [b] Substrate hippuryl-L-arginine at 0.25 and 0.5 mM; enzyme at 2.67×10^{-8} M. [c] Substrate carbobenzyloxyglycylglycylphenylalanine (Byers and Wolfenden, 1973). [d] Substrate hippuryl-L-arginine (Zisapel and Sokolovsky, 1974). [e] Essentially no inhibition at 5 mM.

less in the presence of GEMSA. This decrease was in the nonretarded elution position of the amino acid analyzer column representing acid-unstable material from a glutamic acid alkylation. Similarly, GEMSA protected carboxypeptidase B to a lesser but significant extent from alkylation by [1-^{14}C]-bromoacetamidobutylguanidine. Although some inhibition of the enzyme still occurred, total incorporation of label was significantly reduced. This reduction occurred primarily in *O*-carboxymethyltyrosine.

Effect of Competitive Inhibitors on Other Carboxypeptidases. Inhibitor binding constants for bovine carboxypeptidase A and porcine carboxypeptidase B with BzlSA have been reported and are listed in Table V. We have compared these enzymes with BzlSA and with GEMSA and APMSA. The results (Table V) confirm that BzlSA is an excellent inhibitor of both enzymes. Neither GEMSA nor APMSA has any significant effect on the activity of bovine carboxypeptidase A. Both inhibitors appear somewhat more effective than BzlSA as inhibitors of porcine carboxypeptidase B.

Discussion

Byers and Wolfenden (1973) demonstrated that BzlSA, an analogue of phenylalanine, was the most effective reversible inhibitor known for bovine carboxypeptidase A. Zisapel and Sokolovsky (1974) extended this finding to include porcine carboxypeptidase B because of known similarities in their active sites. We reasoned that by-product analogue compounds based on the structure of arginine and lysine would be more efficient inhibitors for carboxypeptidase B than was BzlSA. Our studies with GEMSA and APMSA (Tables II and V) support this hypothesis. We have shown also (Table V) that GEMSA and APMSA have little inhibitory effect against bovine carboxypeptidase A and are therefore more selective inhibitors than BzlSA. GEMSA and APMSA are the most potent competitive inhibitors yet synthesized for carboxypeptidase B.

GEMSA and APMSA at low ratios of competitive inhibitor to enzyme were effective against alkylating reagents inactivating bovine carboxypeptidase B at two different sites in the active center, namely, at glutamic acid-270 and tyrosine-248 (Table III). These results would be expected for competitive inhibitors that are bound more efficiently to the active center than the alkylating reagents are bound. These results were supported (Table IV) by using radioactive alkylating reagents and observing, as direct evidence of protection of the active-center glutamic acid and tyrosine, lowered production of their radioactive derivatives.

A sulfur atom apparently substitutes adequately for a methylene group in these derivatives. Each of the three all-carbon compounds listed in Table II—guanidinocaproic acid, guanidinovaleric acid, and ε-aminocaproic acid—has a corresponding sulfur-containing analog. These analogues are GEMPA (Ib), GEMAA (Ic), and APMAA (IIc), respectively. The K_is for this group of compounds do not differ by more than a factor of 2 from the all-carbon compounds, with two of the

all-carbon compounds slightly more efficient and the third as efficient a competitive inhibitor.

All dicarboxylic acid compounds studied are D,L mixtures, but K_i values for D- and L-BzlSA have also been reported (Byers and Wolfenden, 1973; Zisapel and Sokolovsky, 1974). Both isomers have inhibitory activity, with the L isomer less than ten times more effective than the D isomer. We attempted to resolve GEMSA by selective trypsin hydrolysis of the dimethyl ester of GEMSA in a manner analogous to that used for BzlSA with chymotrypsin (Byers and Wolfenden, 1972). However, our results were inconclusive.

BzlSA was first proposed as a "by-product analogue" that combines "characteristics of the two products of ester or peptide hydrolysis in a single molecular species" (Byers and Wolfenden, 1973). The proposed structure included both a binding site for the C-terminal carboxyl group and a binding site for the newly formed carboxyl group at the position of cleavage to explain the extreme efficiency of the dicarboxylic acid inhibitors that were spatially four carbons apart. The authors also speculated that BzlSA might supply one ligand to zinc in the enzyme–inhibitor complex.

Cushman et al. (1977) and Ondetti et al. (1977) have carried this study one step further with angiotensin-converting enzyme, a dipeptidyl-carboxypeptidase that is also a zinc metalloenzyme. They initially prepared dicarboxylic acid analogues with K_is of approximately 10^{-6} M. Reasoning that replacement of the second carboxyl function with other groups more capable of supplying a ligand to zinc might give a better inhibitor, they substituted a mercapto group for this second carboxyl function and improved the K_is by 1000-fold. This provides further indirect evidence for the proposition that one ligand binds to zinc in carboxypeptidases A and B. Preparation of the mercapto derivatives of BzlSA, GEMSA, and APMSA would allow this suggestion to be studied further.

Human carboxypeptidase N, a carboxypeptidase B-type enzyme in plasma, functions as a bradykinin (Erdös and Sloane, 1962) and an anaphylatoxin inactivator (Bokisch and Müller-Eberhard, 1970) by releasing C-terminal arginine. GEMSA and APMSA, but not BzlSA, are competitive inhibitors of carboxypeptidase N (Plummer, unpublished observations) and could function as bradykinin potentiators by inhibiting its inactivation. Studies are in progress to test these compounds and to prepare mercapto derivatives that may be even more efficient.

Acknowledgments

We thank Drs. William Lawson and Thomas Ryan for advice on synthetic procedures and Dr. David Wos for discussion on kinetic interpretations. We also thank Mr. Arthur Phelan for excellent technical assistance.

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