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INHIBITION OF SERINE PROTEINASES BY *p*-CARBETHOXYPHENYL ESTERS OF ε-GUANIDINO- AND ε-AMINO CAPROIC ACID: THERMODYNAMIC AND MOLECULAR MODELING STUDY

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The inhibitory effect of the clinically used *p*-carbethoxyphenyl ester of ε-guanidino-caproic acid methanesulphonate (ε-GCA-CEP) on the catalytic properties of human LYS^{77}-plasmin (EC 3.4.21.7), bovine factor Xa (EC 3.4.21.6), bovine α-thrombin (EC 3.4.21.5), ancrod (EC 3.4.21.28), crotalase (EC 3.4.21.30), bovine β-trypsin (EC 3.4.21.4), porcine pancreatic β-kallikrein-B (EC 3.4.21.35), human urinary kallikrein (EC 3.4.21.35) and the M_r 54,000 species of human urokinase (EC 3.4.21.31) was investigated (between pH 2.0 and 8.5, I = 0.1 M; T = 21 ± 0.5°C), and analyzed in parallel with that of the homologous derivative *p*-carbethoxyphenyl ε-amino-caproate hydro chloride (ε-ACA-CEP). On lowering the pH from 5.5 to 3.0, values of the apparent dissociation inhibition constant (K_i) for ε-GCA·CEP and ε-ACA-CEP interaction with the serine proteinases considered increase, reflecting the acidic pK-shift upon inhibitor binding of a single ionizing group. Over the whole pH range explored, (i) ε-GCA-CEP interacts with bovine factor Xa and bovine α-thrombin with an higher affinity than that observed for ε-ACA-CEP binding; (ii) both inhibitors associate to bovine β-trypsin with the same affinity; and (iii) ε-ACA-CEP inhibits human Lys^{77}-plasmin and the M_r 54,000 species of human urokinase with an higher affinity than that reported for ε-GCA-CEP association, thus reflecting the known enzyme primary specificity properties. However, the affinity of ε-ACA-CEP for ancrod, crotalase, porcine pancreatic β-kallikrein-B and human urinary kallikrein, all of which preferably bind arginyl rather than lysyl side chains at the primary position of substrates and/or inhibitors, is paradoxically higher than that displayed by ε-GCA-CEP. By considering the amino acid sequences, the X-ray three-dimensional structures and/or the computer-generated molecular models of serine proteinase: inhibitor adducts, the observed binding behaviour of ε-GCA-CEP and ε-ACA-CEP to the enzymes considered has been related to the inferred stereochemistry of proteinase: inhibitor contact region(s).

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Abbreviations: ε-ACA-CEP, ε-amino caproic acid *p*-carbethoxyphenyl ester chloride; ε-GCA-CEP, ε-guanidino-caproic acid *p*-carbethoxyphenyl ester methanesulphonate (gabexate mesylate, Foy®); ZArgONp, *N*-carbobenzoxy-L-arginine *p*-nitrophenyl ester; ZLysONp, *N*-carbobenzoxy-L-lysine *p*-nitrophenyl ester.

KEY WORDS: ε-guanidino-caproic acid *p*-carbethoxyphenyl ester methanesulphonate ε-amino-caproic acid *p*-carbethoxyphenyl ester chloride; serine proteinase inhibitors, serine proteinase: inhibitor adduct formation (thermodynamics of) serine proteinase: inhibitor adducts (molecular modeling of).

INTRODUCTION

Serine proteinases play a central role in several biological processes, spanning from basic functions in digestion to key regulatory mechanisms such as peptide hormone release, coagulation and complement activation. They are also recognized as pathogenetic factors in many diseases, including tumors, inflammatory processes, pulmonary emphysema, glomerulonephritis, acute pancreatitis and muscolar distrophy.[1-4] Therefore, the possibility of selectively influencing proteinase activities by specific inhibitors appears of considerable interest in view of their therapeutic value as drugs.[5-6]

The *p*-carbethoxyphenyl ester of ε-guanidino-caproic acid methanesulphonate (known also as ε-GCA-CEP, gabexate mesylate and FOY®) is the first non antigenic synthetic proteinase inhibitor used therapeutically in the treatment of pancreatitis as well as disseminated intravascular coagulation, and as a regional anticoagulant agent for hemodialysis.[7-10] The low affinity of ε-GCA-CEP for kallikreins, as compared to that observed for several homologous plasma, pancreatic and urinary trypsin-like enzymes,[10] has been related, on the basis of stereochemical considerations, to unfavourable interaction(s), occurring at the primary specificity subsite (i.e. S_1) of kallikreins, between the proteinase seryl residue, at position 226, and the bulky ε-guanidino aliphatic chain of the inhibitor.[10] The amino acid substitution at this position of the S_1 subsite (serine versus glycine for the other trypsin-like proteinases) is reflected by an increase in affinity for ε-GCA-CEP of about three orders of magnitude.[10]

To gain more information on the inhibitory mechanism of ε-GCA-CEP, the effect of pH (pH 2.0–8.5, I = 0.1M at 21 ± 0.5°C has been investigated on the values of the apparent dissociation inhibition constant (K_i) for the binding of ε-GCA-CEP and its homologous derivative (*p*-carbethoxyphenol ester of the ε-amino-caproic acid chloride; ε-ACA-CEP) to human Lys^{77}-plasmin, bovine factor Xa, bovine α-thrombin, ancrod, crotalase, bovine β-trypsin, porcine pancreatic β-kallikrein-B, human urinary kallikrein and the M_r 54,000 species of human urokinase. Analysis of amino acid sequences,[11-16] three-dimensional structures[14,17-22] and/or of computer-generated molecular models (see References. 10, 23) of serine proteinase:inhibitor adducts has allowed a correlation between the binding behaviour of ε-GCA-CEP and ε-ACA-CEP and the stereochemistry of the enzyme: inhibitor contact region(s).

MATERIALS AND METHODS

Materials

Human Lys^{77}-plasmin was prepared[24] from Lys^{77}-zymogen by activation with urokinase-substituted Sepharose-4B. Bovine factor Xa was obtained from Sigma Chemical CO. (St. Louis, U.S.A.). Bovine α-thrombin was isolated[25] from commercial enzyme preparations (from Sigma Chemical Co., St. Louis, U.S.A.) Ancrod, the coagulating serine proteinase from the Malayan pit viper (*Agkistrodon rhodostoma*) venom, was

purified from commercial enzyme preparations (from Sigma Chemical Co., St. Louis, U.S.A) as reported.[26] Crotalase, the coagulating serine proteinase from the eastern diamondback rattlesnake (*Crotalus adamanteus*) venom, was isolated[27] from commercial enzyme preparations (from Sigma Chemical Co., St. Louis, U.S.A.)

Bovine β-trypsin (treated with diphenylcarbamyl chloride in order to abolish chymotryptic activity) was purified[28] from commercial enzyme preparations (from Sigma Chemical Co., St. Louis, U.S.A.) Neuraminidase-treated porcine pancreatic β-kallikrein-B was isolated[29] from pre-purified enzyme preparations, kindly provided by Bayer AG (Wuppertal, F.R.G.). Neuraminidase-treated human urinary kallikrein,[30] and the 54,000 species of human urokinase[31] were kindly provided by Lepetit S.p.A. (Milano, Italy).

ε-GCA-CEP and ε-ACA-CEP were purchased from Lepetit S.p.A. (Milano, Italy). ZArgONp was synthesized as previously reported.[32] ZLysONP was obtained from Sigma Chemical Co. (St. Louis, U.S.A.). All the other reagents were purchased from Merck AG (Darmstadt, F.R.G.). All chemicals were of analytical grade and used without further purification. The characterization of enzymes, inhibitors and substrates has been reported elsewhere.[9,10,25–35]

Determination of the Apparent Dissociation Inhibition Constant (K_i)

Values of the apparent dissociation inhibition constant (K_i) for ε-GCA-CEP and ε-ACA-CEP binding to the serine proteinases examined were determined, between pH 2.0 and 8.5 (I = 0.1 M) and 21 ± 0.5°C, by assay systems using (i) ZArgONp as substrate for bovine factor Xa, bovine α-thrombin, ancrod, crotalase, porcine pancreatic λ-kallikrein-B and human urinary kallikrein; and (ii) ZLysONp as substrate for human Lys^{77}-plasmin, bovine β-trypsin and the M_r 54,000 species of human urokinase.

In spite of the inactivation of ε-GCA-CEP by human plasma hydrolases ($t_{1/2} \simeq 60$ to 80 s),[9] both ε-GCA-CEP and ε-ACA-CEP were not significantly hydrolyzed (< 5%) by the serum enzymes considered during the time assay (~ 1 min).[7–10]

The detailed biochemical procedures have been published previously.[9,10,35]

Values of K_i were determined using the graphical method reported from Ascenzi *et al.*[36] An average error value of ±8% was evaluated for K_i values according to the method of Sagnella.[37]

Preparation of Computer-Generated Molecular Models

For the preparation of the molecular models mentioned the program FRODO was used throughout[38] in conjunction with an Evans and Sutherland PS300 graphics system.[10,23]

Atomic coordinates for the adducts of bovine (β-)trypsin-(ogen) and porcine pancreatic β-kallikrein-B with benzamidine as well as the native and/or chemically modified bovine basic pancreatic trypsin inhibitor (Kunitz inhibitor)[14,17,18,20,21] were recovered from the Brookhaven Protein Data Bank distribution tape.[39]

Central site models of human Lys^{77} plasmin, bovine factor Xa, bovine α-thrombin and the M_r 54,000 species of human urokinase as well as of human urinary kallikrein were obtained by susbstitution of the relevant amino acid side chains of bovine β-trypsin and porcine pancreatic β-kallikrein-B,[11,14,17,18,20,21] respectively, according to the molecular information available.[11–13,15,16,19,22] The limited molecular data for ancrod and crotalase[26,27,35,40] did not allow the preparation of suitable structures.

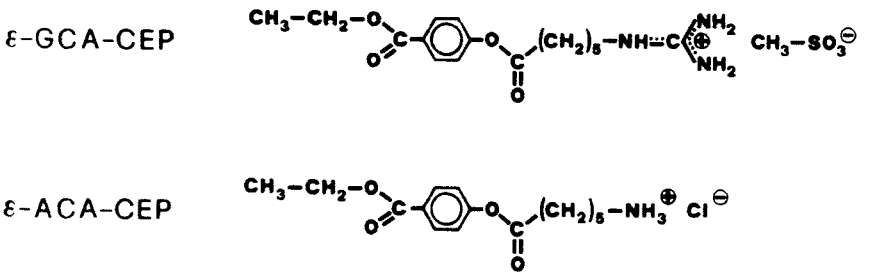

FIGURE 1 Chemical structures of ε-GCA-CEP and ε-ACA-CEP.

The atomic models of ε-GCA-CEP and ε-ACA-CEP were assembled on the basis of their chemical structures (see figure 1), allowing for proper torsional angles, and using tabulated bond lengths and angles.[41]

Both inhibitors ε-GCA-CEP and ε-ACA-CEP were assumed to bind on the S_1 subsite of the serine proteinases considered with their ε-guanidino and ε-amino group, respectively,[10,23] both residues being orientated as observed for the inhibitor amidino, lysyl and arginyl side chains in the adducts of bovine (β-)trypsin(ogen) and porcine pancreatic β-kallikrein-B with benzamidine as well as the native and/or chemically-modified bovine basic pancreatic trypsin inhibitor, respectively.[14,17,18,20,21] Given this location of the ε-guanidino and ε-amino group of ε-GCA-CEP and ε-ACA-CEP respectively, additional possible enzyme: inhibitor interaction(s) were explored directly on the graphics system by considering the torsional degrees of freedom of both inhibitor molecules.

RESULTS AND DISCUSSION

For all enzyme:inhibitor systems examined, the inhibition patterns were strictly competitive, and titrations conformed to simple equilibria. For each proteinase, values of K_i for ε-GCA-CEP and ε-ACA-CEP binding were independent of the enzyme concentration, and compared well with those reported from the literature[7,8,10] if differences in proteinase preparations, inhibitor assay procedure, temperature, as well as pH and/or buffer composition are taken into account (see Figure 2).

Figure 2 shows the effect of pH (between 2.0 and 8.0), at 21 ± 0.5°C, on the affinity (i.e., on K_i values) of ε-GCA-CEP and ε-ACA-CEP for human Lys^{77}-plasmin, bovine factor Xa, bovine α-thrombin, ancrod, crotalase, bovine β-trypsin, porcine pancreatic β-kallikrein-B, human urinary kallikrein and the M_r 54,000 species of human urokinase.

On lowering the pH from 5.5 to 3.0, the increase in the values of K_i for ε-GCA-CEP and ε-ACA-CEP binding to the serine proteinases examined reflects, according to linkage relations,[42] the acidic pK-shift of a single ionizing group upon inhibitor binding. This simple model leads to the following equation (1):

$$\log K_i = C + \log\{([H^+] + 10^{-pK_{UNL}})/([H^+] + 10^{-pK_{LIG}})\} + (pK_{UNL} - pK_{LIG}) \quad (1)$$

Where C is a constant that corresponds to the alkaline asymptote of $\log K_i$, and pK_{UNL}

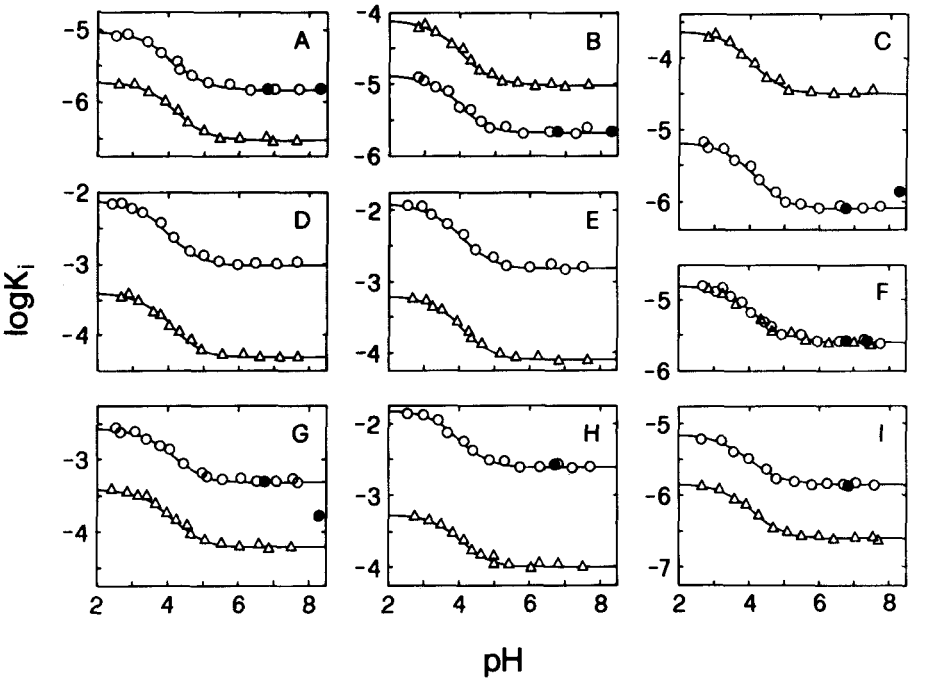

FIGURE 2 pH dependence of the apparent dissociation inhibition constant (K_i, M) for ε-GCA-CEP (○,●) and ε-ACA-CEP (△) binding to human Lys[77]-plasmin (A), bovine factor Xa (B), bovine α-thrombin (C), ancrod (D), crotalase (E), bovine β-trypsin (F), porcine pancreatic β-kallikrein-B (G), human urinary kallikrein (H) and the M_r 54,000 species of human urokinase (I), at 21°C. The continuous lines, calculated from eqn. (1) with values of pK_{UNL} and pK_{LIG} shown in Table 1, were obtained with an iterative non-linear least-squares curve-fitting procedure. Values of K_i for ε-GCA-CEP (●) binding to human plasmin (A), bovine factor xa (B), bovine thrombin (C), bovine trypsin (F), porcine pancreatic kallikrein (G), human urinary kallikrein (H) and human urokinase (I) (T = 20°C to 37°C; Tris [hydroxymethyl]-aminomethane and phosphate buffer systems, I = 0.05 to 0.1 M)[7,8,10] have been included only for comparison; such data have not been used for fitting analysis, since they were obtained under non-homogeneous experimental conditions. The pH profile was explored using the following buffers: phosphate (pH 2.0–2.5); acetate (pH 3.5 to 6.0); and phosphate (pH 6.0–8.0); all at I = 0.1 M (sodium salts). No specific ion effects were found using different buffers with overlapping pH values.

and pK_{LIG} are the pK values of the apparent proton dissociation constants for the inhibitor-free (K_{UNL}) and inhibitor-bound (K_{LIG}) enzymes, respectively. Eqn. (1) has been used to generate the continuous lines shown in Figure 2 with the choice of parameters given in Table 1. In spite of the uncertainty in the evaluation of the acid asymptotes, the agreement with the experimental data is satisfactory (see Figure 2).

Moreover, data shown in Figure 2 indicate that ε-GCA-CEP and ε-ACA-CEP binding (see Table I) are closely similar for the enzymes investigated, and agree very well with the pK values of amino acid residue(s) modulating, between pH 3.0 and 5.5, spectral, inhibitor binding and catalytic properties of the serine proteinases considered, all acting on cationic substrates.[24-27,29,31,33,35,43-47]

Inspection of the amino acid sequences,[11-16] three dimensional structures[14,17-22] and/or computer-generated molecular models[10,23] of serine proteinase:ε-GCA-CEP and:ε-ACA-CEP adducts suggests that the ionizable group affecting inhibitor binding

TABLE I

pK values of the inhibitor-free (pK_{UNL}) and inhibitor-bound (pK_{LIG}) human Lys^{77}-plasmin, bovine factor Xa, bovine α-thrombin, ancrod, crotalase, bovine β-trypsin, porcine pancreatic β-kallikrein-B, human urinary kallikrein and the M_r 54,000 species of human urokinase at 21°C (I = 0.1 M)

Proteinase	ε-GCA-CEP	ε-ACA-CEP
Human Lys^{77}-plasmin	$pK_{UNL} = 4.50$ $pK_{LIG} = 3.70$	$pK_{UNL} = 4.60$ $pK_{LIG} = 3.80$
Bovine factor Xa	$pK_{UNL} = 4.40$ $pK_{LIG} = 3.60$	$pK_{UNL} = 4.50$ $pK_{LIG} = 3.60$
Bovine α-thrombin	$pK_{UNL} = 4.60$ $pK_{LIG} = 3.70$	$pK_{UNL} = 4.55$ $pK_{LIG} = 3.65$
Ancrod	$pK_{UNL} = 4.40$ $pK_{LIG} = 3.50$	$pK_{UNL} = 4.50$ $pK_{LIG} = 3.60$
Crotalase	$pK_{UNL} = 4.55$ $pK_{LIG} = 3.70$	$pK_{UNL} = 4.50$ $pK_{LIG} = 3.60$
Bovine β-trypsin	$pK_{UNL} = 4.55$ $pK_{LIG} = 3.75$	$pK_{UNL} = 4.55$ $pK_{LIG} = 3.75$
Porcine pancreatic β-Kallikrein-B	$pK_{UNL} = 4.50$ $pK_{LIG} = 3.75$	$pK_{UNL} = 4.50$ $pK_{LIG} = 3.70$
Human urinary kallikrein	$pK_{UNL} = 4.40$ $pK_{LIG} = 3.60$	$pK_{UNL} = 4.45$ $pK_{LIG} = 3.70$
The M_r 54,000 species of human urokinase	$pK_{UNL} = 4.40$ $pK_{LIG} = 3.70$	$pK_{UNL} = 4.50$ $pK_{LIG} = 3.75$

pK values were determined by curve-fitting from eqn. (1) to data in Figure 2A–I. An average error value of ± 15% was evaluated for K_{UNL} and K_{LIG} values according to the iterative non-linear least-squares curve-fitting procedure (see Figure 2).

can be identified with the invariant aspartyl residue present at the S_1 subsite of human Lys^{77}-plasmin (at position 735), bovine factor Xa (at position 227), bovine α-thrombin, bovine β-trypsin (see Figures 3A and 4A), porcine pancreatic β-kallikrein-B (see Figures 3B and 4B) and human urinary kallikrein (all at position 189), as well as the M_r 54,000 species of human urokinase (at position 350).[48] In fact, it is known that this residue interacts with the positively charged amidino group of benzamidine, as well as with the cationic ε-amino or δ-guanidino residue of the Lys15 and Arg15 side chain, respectively, of the native and chemically-modified bovine basic pancreatic trypsin inhibitor, in the S_1 subsite of bovine (β-)trypsin(ogen) and porcine pancreatic β-kallikrein-B.[14,17,18,20,21,49] Such stereochemical contribution may also hold in modulating spectral, inhibitor binding and catalytic properties of ancrod and crotalase,[26,27,35] two kallikrein-like serine proteinases acting on cationic substrates,[35] as suggested from the limited molecular information available.[26,27,35,40]

Over the whole pH range explored (2.0 to 8.5), the affinity of ε-GCA-CEP for the serine proteinases considered does not strictly follow the primary specificity of the enzymes for the ε-amino or the δ-guanidino group of lysyl or arginyl residues at the primary position (i.e., P_1 of substrates and/or inhibitors (see Figure 2). In fact, on the basis of the primary specificty of the proteinases, ε-GCA-CEP should show strong

affinity for bovine factor Xa, bovine α-thrombin, ancrod, crotalase, porcine pancreatic β-kallikrein-B and human urinary kallikrein, all of which preferentially bind argynyl side chains,[25–27,29,30,33,35,44,47] intermediate affinity for bovine β-trypsin, which does not discriminate between the two cationic residues,[44,47] and weak affinity for human Lys[77]-plamin and the M_r 54,000 species of human urokinase, both of which preferentially interact with lysyl side chains.[24,31,44,45] Instead, on the basis of the K_i values, the affinity of ε-GCA-CEP to the serine proteinases considered can be arranged as follows: human urinary kallikrein ~ ancrod ~ crotalase ~ porcine pancreatic β-kallikrein-B ≪ bovine β-trypsin ~ bovine factor Xa ~ human Lys[77]-plasmin ~ the M_r 54,000 species of human urokinase ~ bovine α-thrombin. On the other hand, data given in Figure 2 indicate that the affinity of ε-ACA-CEP for the serine proteinases considered follows the primary specificity of the enzymes investigated for the ε-amino group of the lysyl side chain. Thus, ε-ACA-CEP shows highest affinity for the M_r 54,000 species of human urokinase and human Lys[77]-plasmin, intermediate affinity for bovine β-trypsin, and weak affinity for bovine factor Xa,

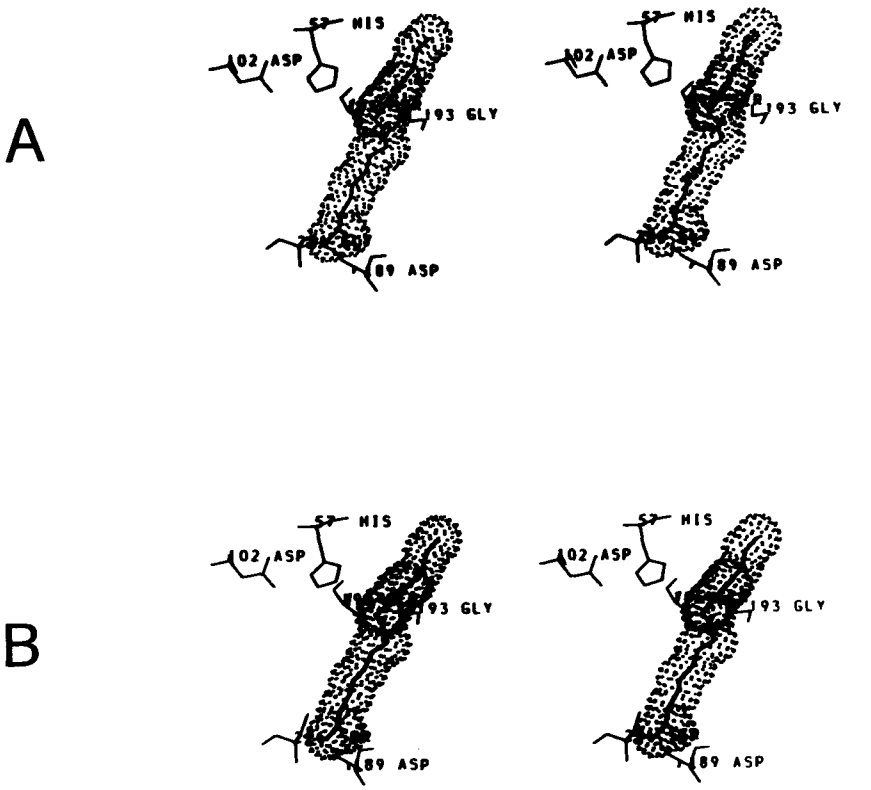

FIGURE 3 Stereoviews of the close chemical environment of ε-GCA-CEP in its adducts with bovine β-trypsin (A) and porcine pancreatic β-kallikrein-B (B). In trypsin-like proteinases, the residue at position 772 in human Lys[77]-plasmin, 264 in bovine factor Xa, 226 in bovine α-thrombin, as well as bovine β-trypsin, and 387 in the M_r 54,000 species of human urokinase is a glycine.[11–13,15,17–20,22] In kallikrein-like proteinases, namely porcine pancreatic β-kallikrein-B, human urinary kallikrein and possibly ancrod as well as crotalase, position 226 is occupied by a serine residue.[14,16,21] Dots represent the van der Waals surface of ε-GCA-CEP.

bovine α-thrombin, ancrod, porcine pancreatic β-kallikrein-B, crotalase and human urinary kallikrein.

Moreover, data shown in Figure 2 indicate that bovine factor Xa and bovine α-thrombin bind preferentially ε-GCA-CEP rather than ε-ACA-CEP, bovine β-trypsin shows the same affinity for both inhibitors, and human Lys^{77}-plasmin as well as the M_r 54,000 species of human urokinase interact preferentially with ε-ACA-CEP rather than with ε-GCA-CEP; on the other hand, ancrod, crotalase, porcine pancreatic β-kallikrein-B and human urinary kallikrein show an unexpected higher affinity for ε-ACA-CEP rather than for ε-GCA-CEP.

Stereochemical considerations based on inspection of the computer-generated models of the proteinase:ε-GCA-CEP and:ε-ACA-CEP adducts (see Figures 3 and 4) suggest that the source of different inhibitor selectivity probably resides in the length of the aliphatic chain connecting the ε-guanidino or the ε-amino groups. In fact, comparison of an arginine residue with ε-GCA-CEP shows that the inhibitor is one carbon atom longer in its aliphatic chain. This stereochemical constraint has the effect of pushing the bulky ε-guanidino group somewhat deeper into the primary specificity pocket, and/or of requiring more room, along the pocket itself. On this basis, the amino acid residues at the entrance and within the S_1 subsite may play a determinant role in affecting inhibitor binding. In this respect, it is worth noting that the residue

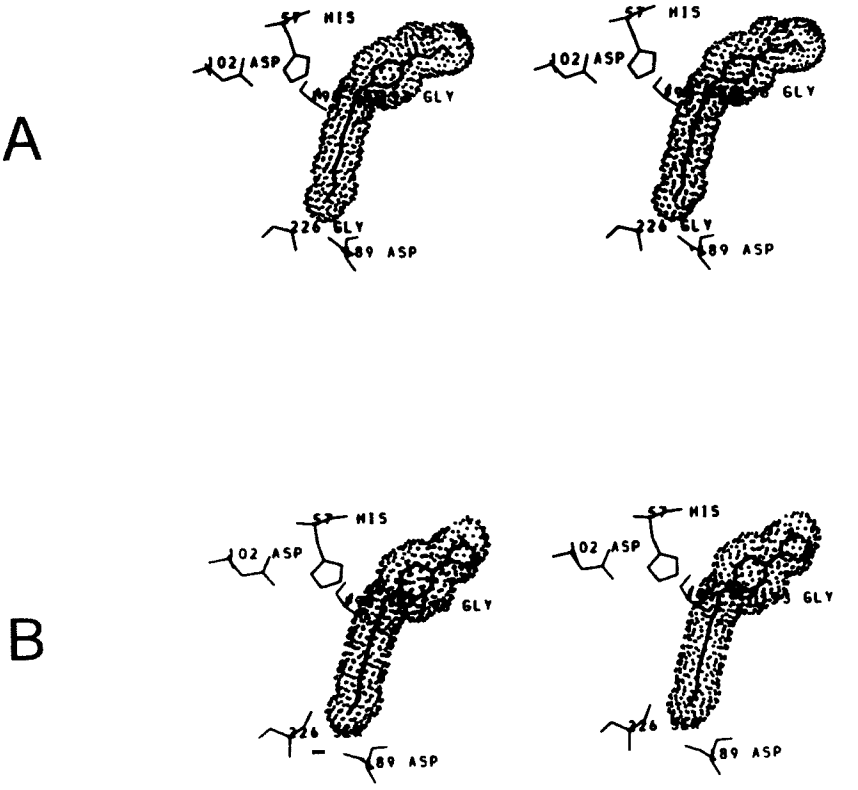

FIGURE 4 Stereoviews of the close chemical environment of ε-ACA-CEP in its adducts with bovine β-trypsin (A) and porcine pancreatic β-kallikrein-B (B). For details see Figure 3.

at position 772 in human Lys[77]-plasmin, 264 in bovine factor Xa, 226 in bovine α-thrombin as well as bovine β-trypsin (see Figures 3A and 4A), and 387 in the M_r 54,000 species of human urokinase is glycine.[11–13,15,17–20,22] However, residue 226, the side chain of which points to the interior of the S_1 subsite (at its bottom), is serine in porcine pancreatic β-kallikrein-B (see figures 3B and 4B), human urinary kallikrein and possibly in the homologous proteinases ancrod and crotalase.[14,16,21,26,27,35,40] Model building indicates that unfavourable interatomic (enzyme:inhibitor) contacts, arising from the presence of residues bulkier than glycine at this position, can be a determining factor in modulating enzyme interaction(s) with an ε-guanidino aliphatic chain longer than an arginyl residue. Minor structural sources of proteinase: ε-GCA-CEP and:ε-ACA-CEP adducts (de)stabilization at the S_1 subsite (such as a slightly different polypeptide backbone conformation and the shift or removal of tightly bound water molecules) cannot be ruled out on the basis of this analysis. Such interpretation is in line with the evidence for a critical correlation between the inhibitory as well as catalytic quantities and side chain length in serine proteinase action.[23,44–47,50]

The present modeling study suggests that common additional proteinase: ε-GCA-CEP and:ACA-CEP stabilization factors arise also from the hydrogen bonds occurring at the catalytic "oxyanion site" between the amide hydrogen atoms of invariant glycyl and seryl enzyme residues (respectively at positions 739 and 741 in human Lys[77]-plasmin, 231 and 233 in bovine factor Xa, 193 and 195 in bovine α-thrombin, bovine β-trypsin, porcine pancreatic β-kallikrein-B, human urinary kallikrein as well as possibly in the homologous proteinases ancrod and crotalase, and 354 and 356 in the M_r 54,000 species of human urokinase[11,22,26,27,35,40] and the carbonyl oxygen of the ester bridge connecting the *p*-carbethoxyphenyl group with the ε-guanidino-or ε-amino-caproic acid aliphatic chain. In this way the scissile ester linkage falls in a position identical to that occupied by the "reactive" peptide bond of proteinase inhibitors (and possibly substrates) with respect to the Asp-His-Ser catalytic triad of serine proteases.[23] This finding may indeed be the structural basis of ε-GCA-CEP hydrolysis catalyzed by human plasma hydrolases.[7–10]

Next, from stereochemical observations, it was not possible to find a preferential orientation for the *p*-carbethoxyphenyl moiety of ε-GCA-CEP and ε-ACA-CEP corresponding to productive interaction(s) with the surface of the enzymes; it is therefore likely that this part of the inhibitor molecule does not specifically recognize the various proteinases and possibly adopts in time different conformations.

As a whole, these data indicate that the optimal fit of ε-GCA-CEP and ε-ACA-CEP at the central site of the serine proteinases considered depends primarily on specific interactions with amino acid residues provided by the single enzymes at their S_1 subsite.

Finally, in consideration of the clinical uses of ε-GCA-CEP (FOY) in the treatment of pancreatitis as well as disseminated intravascular coagulation and as a regional anticoagulant for hemodialysis,[7,8,10] its low affinity for kallikreins and related enzymes could be relevant. In fact, it is possible that the selective inhibition of serine proteinases involved in the coagulation system could occur without influencing blood pressure, which is controlled by the kininogen-to kinin conversion catalyzed by kallikreins and related proteinases.[29,35]

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48. Amino acid residues have been identified by their three-letter code and by their sequence number. The numbering of human Lys^{77}-plasmin refers to that of the complete form (Glu^{1}) of human plasminogen.[13] The numbering of bovine factor xa refers to that of its heavy chain.[12] The numbering of bovine α-thrombin, bovineβ-trypsin, porcine pancreatic β-kallikrein-B and human urinary kallikrein refers to that of bovine chymotrypsinogens A and B.[11] The numbering of the M_r 54,000 species of human urokinase refers to that of the single-chain pro-urokinase.[15]
49. The pK values of the ε-guanidino and ε-amino group of ε-GCA-CEP and ε-ACA-CEP, respectively, are higher than 10; therefore, over the whole pH range explored (2.0 to 8.5), the inhibitors examined are always in the cationic form.[10]
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