Rat Insulin-Degrading Enzyme: Cleavage Pattern of the Natriuretic Peptide Hormones ANP, BNP, and CNP Revealed by HPLC and Mass Spectrometry†

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ABSTRACT: The degradation of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) by insulin-degrading enzyme (IDE) has been investigated. As revealed by high-performance liquid chromatography, all three peptides are sequentially cleaved at a limited number of sites, the latter of which were identified by mass spectrometric analyses. The studies revealed that ANP is preferred as substrate over BNP and CNP. ANP degradation is rapidly initiated by hydrolysis at the Ser^{25}–Phe^{26} bond. Three additional cleavage sites were identified in ANP after prolonged incubation with IDE; in contrast, three and two bonds were hydrolyzed in BNP and CNP, respectively. Analysis of the nine cleavage sites shows a preference for basic or hydrophobic amino acid residues on the carboxyl side of a cleaved peptide bond. In contrast to most of the peptide fragments generated by IDE activity, the initial ANP cleavage product, F-R-Y, is rapidly degraded further by cleavage of the R–Y bond. Cross-linking studies with ^{125}I-ANP in the presence of sulfhydryl-modifying agent indicate that IDE activity is inhibited at the level of initial substrate binding whereas metal-ion chelating agents only prevent hydrolysis. On the basis of its structural and enzymatic properties, IDE exhibits striking similarity to a number of recently-described endopeptidases.

Insulin-degrading enzyme (IDE,[1] EC 3.4.22.11) is a cellular metalloendopeptidase that is involved in the proteolysis of internalized insulin (Duckworth, 1988, 1990). Microinjected anti-IDE antibodies inhibit insulin degradation in hepatoma cells (Shii & Roth, 1987). The in vivo insulin cleavage sites are identical to those recognized by in vitro purified IDE (Duckworth et al., 1988, 1990). The recent characterization of IDE-encoding cDNAs from both man (Affholter et al., 1988) and *Drosophila* (Kuo et al., 1990) has revealed highly conserved primary structures of the enzyme. Some fundamental questions remain concerning the biological function and enzymatic properties of this nonlysosomal (Akiyama et al., 1988) protease. For example, the intracellular site of IDE-catalyzed proteolysis is not clear. Although the enzyme has been characterized as a cytosolic protein (Hari et al., 1987), most recent reports refer to IDE activity within vesicles that arise during receptor-mediated endocytosis (Hamel et al., 1991). The function of IDE may be restricted to hormone clearance (Yonezawa et al., 1989), but specific cleavage of substrates to peptide intermediates could also generate new biologically-active molecules (Rose et al., 1988; Steiner, 1977). A regulatory role for IDE has been assumed since the enzyme is apparently involved in the morphological and biochemical differentiation of cultured myoblasts (Kayalar & Wong, 1989) and its gene is developmentally regulated in *Drosophila* (Stoppelli et al., 1988). The enzyme may have cellular substrates other than insulin, since the growth factors TGFα and EGF bind to it with high affinity, and TGFα is selectively degraded by this protein (Garcia et al., 1989).

Finally, the elucidation of the IDE cleavage sites in insulin revealed a peptide bond specificity that was dissimilar to those of other known proteases (Duckworth et al., 1988; Stentz et al., 1989); the enzyme appeared to recognize specific secondary structures rather than a particular amino acid sequence (Stentz et al., 1989). Sequence comparison with other proteases places IDE in a new class of metalloendopeptidases together with *Escherichia coli* protease III and mitochondrial processing endopeptidases (Rawlings & Barrett, 1991).

Atrial natriuretic peptide (ANP) is a high-affinity (k_d = 60 nM) IDE substrate (Müller et al., 1991) and, like insulin, is internalized via receptor-mediated endocytosis (Smith et al., 1988). Both peptide hormones bind to receptors that have a single membrane-spanning domain, and signal transduction occurs through the stimulation of an intrinsic intracellular activity that is either a tyrosine kinase (insulin receptor) or a guanylate cyclase (ANP receptor). A particular feature of the ANP system is the presence of an additional receptor (ANP-C or clearance receptor) that lacks enzymatic activity (Fuller et al., 1988). Binding and internalization of ANP by this receptor are regarded as a clearance mechanism for the hormone (Maack et al., 1987), but little is currently known about the metabolic pathway of ANP following internalization.

A cellular protease that specifically removes the carboxy-terminal tripeptide from ANP has been demonstrated in cell cultures (Johnson et al., 1989, 1990); the enzyme was partially purified and found to be inhibited by metal-ion chelators and sulfhydryl-modifying agents (Johnson & Foster, 1989). Since the sensitivity of these agents is also characteristic of IDE, we suggested that the described ANP-degrading activity was due to IDE or a closely-related enzyme (Müller et al., 1991).

In this study, we elucidate the IDE cleavage site(s) in ANP using HPLC and mass spectrometry. The related natriuretic peptides BNP and CNP, which are structurally very similar, were included to elucidate the substrate specificity of IDE.

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[1] Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid; HPLC, high-performance liquid chromatography; IDE, insulin-degrading enzyme (EC 3.4.22.11); PCMB, *p*-(chloromercuri)benzoate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid.

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EXPERIMENTAL PROCEDURES

Enzyme Preparation and Substrates. Insulin-degrading enzyme was purified from the soluble fraction of rat brain homogenate as described previously (Müller et al., 1991). In brief, the purification steps included ammonium sulfate precipitation, gel filtration and chromatography on Sephacryl S-200 and Mono Q HPLC columns, respectively, and hydrophobic interaction chromatography (HIC).

Further purification of rat IDE was achieved by Sephacryl S-200 refiltration and HIC rechromatography, giving rise to enzyme preparations of at least 60% purity. It should be noted that ANP-binding and ANP-degrading activities were strictly correlated in the elution profiles of each chromatographic step, indicating that the protease activity resides in IDE. Furthermore, a cDNA encoding rat IDE has been identified by screening a rat brain library (unpublished results). The deduced amino acid sequence, which is about 95% identical to the human counterpart, matches the sequences of tryptic peptides obtained from the purified IDE preparations (Müller et al., 1991).

The synthetic substrates rat ANP (rANP, 28 residues), porcine BNP (pBNP, 26 residues), and porcine CNP (pCNP, 22 residues) were purchased from Bissendorf Biochemicals (Hannover, FRG). The purity of the substrates was verified by HPLC analysis (see below). Each peptide preparation gave a single peak with retention times of 32.0 min (ANP) or 34.5 min (BNP and CNP). ^{125}I-ANP (rat, residues 99–126), 2 kCi/mmol, was obtained from Amersham (Braunschweig, FRG).

Degradation of ANP, BNP, and CNP. Reactions were carried out in the presence of 2 nmol of peptide and 14.5 μL of IDE (29 ng of protein) in a total volume of 80 μL at 37 °C. The incubation buffer was 20 mM sodium phosphate, pH 7.2, containing 2 mM $MgCl_2$. At various times, 10-μL aliquots were withdrawn, and degradation was stopped by the addition of 20 μL of trifluoroacetic acid (TFA, 0.1% in H_2O) and immediate freezing in liquid nitrogen. The previous finding that degradation of ^{125}I-ANP is not completely inhibited by excess of unlabeled ANP or insulin is explained by the prolonged incubation time (1 h) used in those experiments (Müller et al., 1991); shorter incubation times (5–15 min) resulted in 100% inhibition of ^{125}I-ANP degradation by either cold ANP or insulin.

HPLC Analysis. HPLC profiles of the cleavage products obtained after 1, 2.5, 5, 15, 22.5, 30, 60, 120, and 240 min were used to investigate the time course of proteolysis. The samples (30 μL) were injected into an Applied Biosystems HPLC system (Model 130 A) connected to a Hewlett Packard 1040 M photodiode array detector, and run on a reversed-phase column (Vydac C18, pore size 300 Å, particle size 5 μm; column dimensions 100 × 2.1 mm with a 20 × 2.1 mm guard column) at a flow rate of 0.2 mL/min with 0.1% TFA in H_2O (solvent A) and 70% acetonitrile/0.08% TFA in H_2O (solvent B); gradient steps were 10 min isocratic 0% B, 0% B to 80% B in 45 min, and 80% B to 100% B in 5 min.

Half-life ($t_{1/2}$) values for ANP, BNP, and CNP were derived from peak areas (monitoring wavelength 214 nm) calculated, using HP Chem Station integration software, from chromatograms at 1-, 2.5-, 5-, 15-, 30-, 60-, and 120-min incubation time.

Mass Spectrometry and Peptide Sequencing. Molecular masses were determined on a Biolon 20 plasma desorption mass spectrometer (Biolon Nordic AB, Uppsala, Sweden). The samples, 30 pmol, were dissolved in 0.1% TFA/20% methanol, placed on aluminized nylon foil coated with

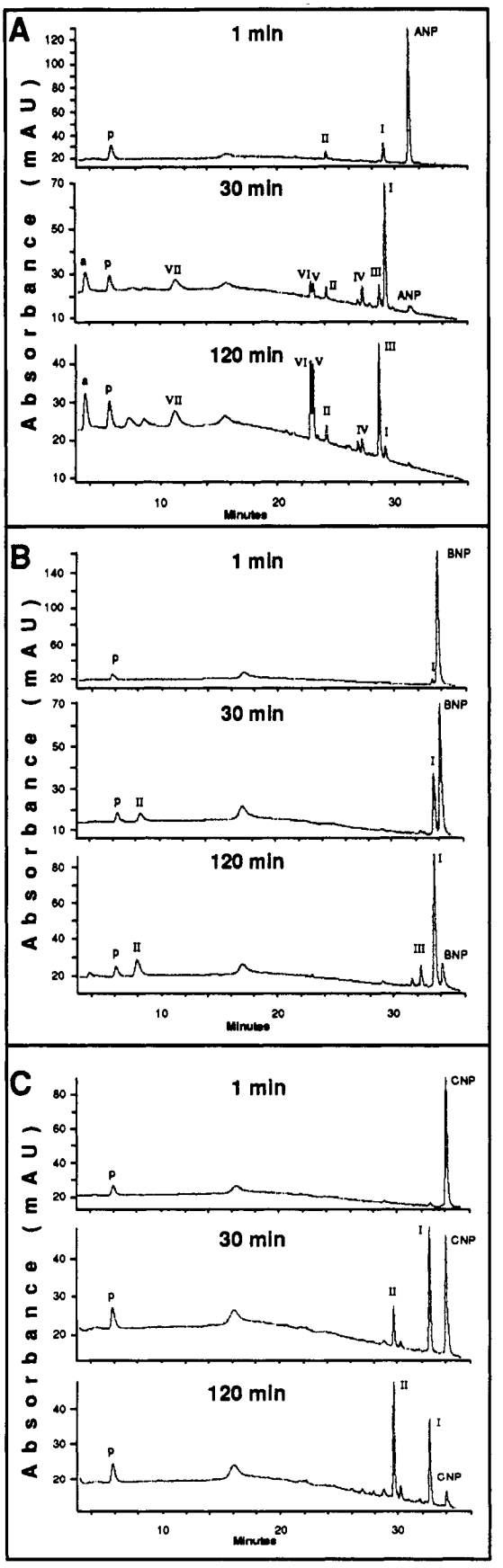

FIGURE 1: HPLC profiles of ANP (A), BNP (B), and CNP (C) after degradation by IDE. The retention times of the peptide fractions are (in minutes) 29.3 (I), 24.3 (II), 28.9 (III), 27.4 (IV), 23.2 (V), 23.0 (VI), 11.2 (VII), and 3.8 (a) in (A), 33.9 (I), 8.5 (II), and 32.7 (III) in (B), and 33.1 (I) and 30.0 (II) in (C). The protease preparation itself gave rise to an absorbance peak (peak p) that eluted after 5.8 min.

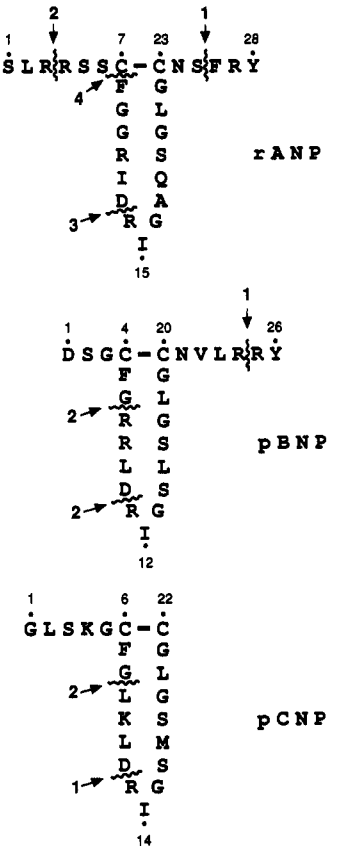

FIGURE 2: Cleavage sites for IDE in the natriuretic peptides. Arrows mark the hydrolyzed peptide bonds in rat (r) ANP and porcine (p) BNP and CNP. The sequence of cleavage events in each peptide is indicated by numbering.

nitrocellulose, and washed with 2 μL of 0.1% TFA if required. An acceleration voltage of 18 kV was applied; spectra from 1 × 106 primary ion events were accumulated. On-probe reductions of disulfide bonds were accomplished by adding 2 μL of 1 M dithiothreitol for 1 h. In routine operations, an accuracy of better than ±2 Da can be expected for average molecular masses. Sequencing of peptide was performed in an automated gas-phase sequencer (Applied Biosystems, Model 470A), equipped with an on-line 120A PTH analyzer.

RESULTS

Degradation of ANP. The cleavage products of the natriuretic peptides generated by the rat insulin-degrading enzyme (IDE) were analyzed by HPLC and mass spectrometry. ANP degradation showed a more complex product pattern and a faster initial hydrolysis (kinetic analyses based on the disappearance of ANP revealed an initial degradation rate of 4 μmol min^{-1} mg^{-1} and a half-life, $t_{1/2}$, of 8 min) than those of either BNP ($t_{1/2}$ = 24 min) or CNP ($t_{1/2}$ = 25 min) (Figure 1A–C). Degradation of ANP by IDE occurred in the following order (Figure 2): (i) hydrolysis of the S^{25}–F^{26} bond, resulting in the long-lived intermediate S^1–S^{25}; (ii) cleavage between R^3 and R^4, leading to a second intermediate (R^4–S^{25}); (iii) hydrolysis of the D^{13}–R^{14} bond; the resulting peptide is further degraded into a hexapeptide (F^8–D^{13}) and a hexadecapeptide, [R^4–C^7]–[R^{14}–S^{25}], revealing a fourth cleavage site between C^7 and F^8.

Cleavage Step 1. The initial degradation products of ANP, after an incubation time of 1 min (peptide fractions I and II, Figure 1A), have protonated molecular ions $(M+H)^+$ at m/z 2597.1 Da (I) and 485.7 Da (II). These values correspond to the calculated size of 2596.9 Da for the ANP fragment S^1–S^{25} and 485.6 Da for the remaining carboxy-terminal tripeptide F^{26}–Y^{28} (Table I). Thus, ANP degradation is rapidly initiated by cleavage between S^{25} and F^{26} (Figure 2), thereby releasing the C-terminal tripeptide F-R-Y.

Table I: Masses and Assigned Structures of the Cleavage Products Generated by IDE[a]

fraction	mass (Da) measured	mass (Da) calculated	assigned structure
		ANP	
I	2597.1	2596.9	S^1–S^{25}
II	485.7	485.6	F^{26}–Y^{28}
III	2240.2	2240.5	R^4–S^{25}
IV	2259.6	2258.5	[R^4–S^{25}] + H_2O
+DTT	1098.2	1097.3	R^4–D^{13}
+DTT	1163.6	1162.4	R^{14}–S^{25}
V	665.1	664.7	F^8–D^{13}
VI	1613.0	1612.8	[R^4–C^7] – [R^{14}–S^{25}]
	1162.2	1163.3	R^{14}–S^{25}
	756.5	756.8	[R^4–C^7] – [C^{23}–S^{25}]
VII	452.0	452.5	R^4–C^7
		BNP	
I	2549.5	2549.9	D^1–R^{24}
II	338.7	338.4	R^{25}–Y^{26}
III	2027.4	2028.3	[D^1–G^6] – [R^{11}–R^{24}]
	1444.2	1445.7	R^{11}–R^{24}
		CNP	
I	2216.8	2216.6	[G^1–C^{22}] + H_2O
+DTT	1238.2	1238.5	G^1–D^{12}
+DTT	981.0	981.2	R^{13}–C^{22}
II	1745.9	1747.1	[G^1–G^8] – [R^{13}–C^{22}]
+DTT	981.1	981.2	R^{13}–C^{22}
+DTT	769.0	768.9	G^1–G^8

[a] The peptide fractions were monitored according to Figure 1. Masses are given as protonated molecular ions, $(M + H)^+$ (see Experimental Procedures), and average molecular masses are used for calculations. Where indicated, DTT was used to reduce disulfide bonds. The assigned structures were verified by gas-phase Edman sequencing (data not shown).

To analyze further the fate of the tripeptide F-R-Y, ^{125}I-ANP, labeled at Y^{28}, was used as a substrate. The labeled cleavage products F-R-Y, R-Y, and Y were obtained regardless of the presence either of the protease inhibitors leupeptin (Figure 3), amastatin, bestatin, or pepstatin A (not shown) or of phosphoramidon, a specific inhibitor of another metalloendopeptidase (endopeptidase 24.11, EC 3.4.24.11) which is known to hydrolyze ANP (Johnson et al., 1990). However, agents that have been shown to inhibit IDE activity like the sulfhydryl-modifying *p*-(chloromercuri)benzoate (PCMB) and the metal-ion-chelating EDTA prevented (PCMB) or strongly inhibited (EDTA) the release of the C-terminal tripeptide as well as the formation of tyrosine (Figure 3). The data indicate that cleavage at the C-terminus of ANP and subsequently of the tripeptide F-R-Y is catalyzed by one and the same enzyme.

Cleavage Step 2. After 30-min incubation, more than 90% of the ANP was degraded, and the initial degradation product, S^1–S^{25}, was converted into peptide fraction III which corresponds to fragment R^4–S^{25}. Thus, the second hydrolysis step occurs at the amino terminus of ANP between R^3 and R^4 (cleavage site 2, Figure 2). The tripeptide S^1–R^3 could not be identified.

Cleavage Step 3. Analysis of fraction IV revealed an $(M+H)^+$ of 2259.6 Da. This size fits well with that of fragment R^4–S^{25} if it contains one hydrolyzed peptide bond within the disulfide-linked loop. After reduction of this disulfide bond with DTT, $(M+H)^+$ ions at m/z 1098.2 Da and m/z 1163.6 Da were found, corresponding to fragments R^4–D^{13} and R^{14}–S^{25}. Hence, R^4–S^{25} (fraction III) must have been converted

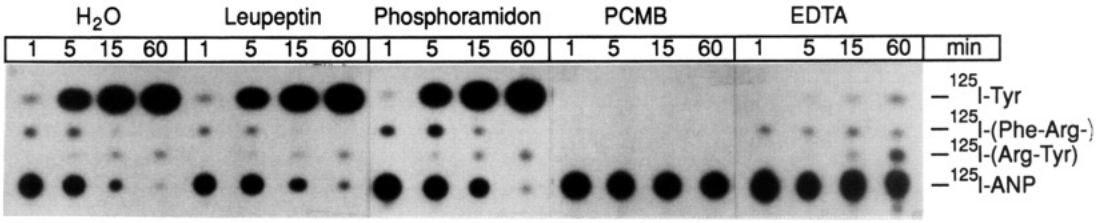

FIGURE 3: Effect of inhibitors on the degradation of ^{125}I-ANP by IDE. Reactions were performed at 25 °C. The enzyme (1.5 ng) was preincubated in 3 μL of 20 mM Hepes, pH 7.5, for 5 min in the presence of either leupeptin (20 μg/mL), phosphoramidon (0.1 mM), *p*-(chloromercuri)benzoate (200 μg/mL), or EDTA (10 mM) or in the absence of inhibitors. Subsequently, the substrate (120 pmol of unlabeled ANP and 10 fmol of ^{125}I-ANP) together with additional inhibitor was added to give a final volume of 12 μL of 20 mM Hepes, pH 7.5, 1 mM DTT, and 5 mM $MnCl_2$. At the times indicated, 2.5 μL was removed, and the reaction was stopped by the addition of 1 μL of a solution containing 25 mM EDTA, 2 mM 1,10-phenanthroline, and 5 mM *N*-ethylmaleimide. Samples were analyzed by thin-layer chromatography (Müller et al., 1991). After a run time of 125 min, the sheets were dried and exposed to X-ray films. The positions of ^{125}I-labeled F-R-Y, R-Y, and Y were verified using reference molecules.

to fraction IV by cleavage between D^{13} and R^{14} (ANP cleavage site 3, Figure 2).

Cleavage Step 4. Fractions V and VI seem to emerge simultaneously; fraction V contains fragment F^8–D^{13}, indicating a further cleavage site within the disulfide-linked loop (ANP cleavage site 4, Figure 2). Consistent with this assumption, peptide VI is found to correspond to the remaining disulfide bond-connected part of R^4–S^{25}, namely, [R^4–C^7]–[R^{14}–S^{25}]. The peptide fraction VI contains two additional $(M+H)^+$ ions of much lower intensity, at m/z 1162.2 Da and m/z 756.5 Da. The former was assigned to fragment R^{14}–S^{25}, presumably originating from [R^4–C^7]–[R^{14}–S^{25}] by reduction of the disulfide bond. The latter corresponds to [R^4–C^7]–[C^{23}–S^{25}], pointing to an additional cleavage site between G^{22} and C^{23} (data not shown). Finally, fraction VII contains the tetrapeptide R^4–C^7, thus confirming that the disulfide bond was reduced during the course of the incubation.

Cleavage Sites in BNP and CNP. Using BNP as substrate, fractions I and II were obtained (Figure 1B) which contain the peptide fragments D^1–R^{24} and R^{25}–Y^{26}, respectively, indicating that degradation is initiated by hydrolysis of the R^{24}–R^{25} bond (BNP cleavage site 1, Figure 2). While the C-terminal fragment R^{25}–Y^{26} was resistant to hydrolysis, the peptide D^1–R^{24} was further degraded, giving rise to a third fraction, that emerged later, containing $(M+H)^+$ ions at m/z 2027.4 Da and, of lower intensity, at m/z 1444.2 Da. The former was assigned to the disulfide bond-connected [D^1–G^6]–[R^{11}–R^{24}], thereby indicating the removal of four amino acid residues from the molecule's loop due to hydrolysis of the peptide bonds between G^6–R^7 and D^{10}–R^{11}. The 1444.2-Da mass was assigned to fragment R^{11}–R^{24}, indicative of a partial reduction of the disulfide bond during the course of the incubation.

Using CNP as substrate for IDE, the two peptides G^1–D^{12} and R^{13}–C^{22} were identified in fraction I (Figure 1C) after reduction of the disulfide bond, revealing a cleavage site between residues D^{12} and R^{13} (Figure 2). Fraction II contains the disulfide bond-connected peptide [G^1–G^8]–[R^{13}–C^{22}], pointing to a second cleavage site within the loop of CNP between residues G^8 and L^9 (CNP cleavage site 2, Figure 2). A minor, uncharacterized peak emerging with a retention time of 30.3 min may represent the excised tetrapeptide L^9–D^{12}.

The first cleavage product of CNP ([G^1–D^{12}])–([R^{13}–C^{22}]) is a rather stable intermediate that is the most prominent peptide after 30-min incubation. Its relative resistance to further hydrolyzation is remarkable when compared to the degradation of BNP, in which the corresponding intermediate with one split bond in the loop is rapidly cleaved at a second site.

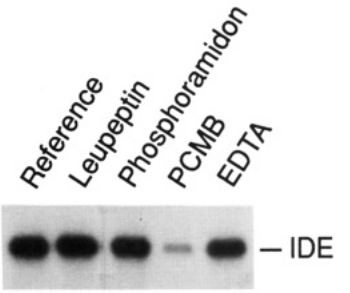

FIGURE 4: Effect of inhibitors on the cross-linking of ^{125}I-ANP to IDE. The enzyme (1.0 ng) was preincubated in the absence or presence of inhibitors as described in the legend to Figure 3. Subsequently, 10 fmol of ^{125}I-ANP, additional inhibitor, and binding buffer were added to give a final volume of 10 μL of 20 mM Hepes, pH 7.5, 1 mM DTT, 2 mM 1,10-phenanthroline, and 150 mM NaCl. After 7 min at 25 °C, 2 μL of the cross-linking reagent bis(succinimidyl suberate) (7.5 mM) was added and the incubation continued for 10 min. Reactions were stopped by adding 5 μL of buffer containing 375 mM Tris-HCl, pH 6.8, 30 g/L SDS, 20% (by volume) glycerol, 200 mM DTT, and 0.6 g/L bromophenol blue. Samples were heated for 1 min at 100 °C and analyzed by SDS–PAGE according to Laemmli (1970) in 6% gels followed by autoradiography.

Discrimination between Substrate Binding and Hydrolysis. *p*-(Hydroxymercuri)benzoate inhibits the binding of ^{125}I-insulin to immunoabsorbed human IDE (Ogawa et al., 1992), suggesting that this inhibitor may affect a certain motif of the insulin-binding site on IDE. It was of interest to examine whether the same holds for the molecular interaction between ^{125}I-ANP and rat IDE. Figure 4 shows that *p*-(chloromercuri)-benzoate (PCMB) strongly inhibits the cross-linking of ^{125}I-ANP to IDE, confirming that this agent affects substrate binding by IDE. In contrast, the presence of EDTA (10 mM), which likewise strongly inhibits the degradation of ^{125}I-ANP, had no influence on the amount of the cross-linked peptide.

These findings suggest that substrate binding and hydrolysis are distinguishable events in the course of IDE action. The cross-linking studies in the presence of ANP show that mercuribenzoate derivatives inhibit IDE activity at the level of substrate binding. In contrast, inhibitors that chelate metal ions, like EDTA (Figure 3) and 1,10-phenanthroline (not shown), do not affect substrate binding. These agents presumably exert their effect by the removal of divalent cations that are required for hydrolysis. An inhibition of peptide degradation thus may account for the reported apparent increase in insulin binding to human IDE in the presence of 1,10-phenanthroline (Ogawa et al., 1992).

DISCUSSION

Recently we identified the rat IDE as an ANP-hydrolyzing enzyme (Müller et al., 1991). Like insulin, ANP has a high

affinity for IDE (Müller et al., 1991), and both peptides apparently compete for the same binding sites. An ANP-degrading enzyme that is specifically inhibited by metal-ion chelators and sulfhydryl-modifying agents (Johnson & Foster, 1990; Stephenson & Kenny, 1987) has been shown to cleave ANP between residues S^{25} and F^{26}, hence removing the C-terminal tripeptide from ANP. On the basis of their strikingly similar enzymatic properties, and the finding that ANP is an IDE substrate, we recently suggested that these studies may have described IDE or an enzyme closely related to IDE (Müller et al., 1991). This is even more supported by the finding that ANP is primarily cleaved at the S^{25}–F^{26} bond.

Comparison of the nine bonds in the natriuretic peptides hydrolyzed by IDE (Figure 2) reveals that six have a basic (arginine) and three a hydrophobic residue (phenylalanine or leucine) on their carboxyl side. Thus, IDE might preferentially catalyze the hydrolysis of bonds that have a basic amino acid residue on the carboxyl side but also tolerate hydrophobic residues at that position. In contrast, the residues identified on the amino side of the hydrolyzed bonds (arginine, serine, cysteine, aspartic acid, and glycine) do not reveal any definite structural requirement for the enzyme. The reported cleavage sites in insulin are not inconsistent with such a preference of IDE. The insulin domain exposed to IDE (Stentz et al., 1989) contains no basic residues; however, a preferential cleavage of bonds with hydrophobic residues (leucine and tyrosine) on the carboxyl side is observed (Duckworth et al., 1988; Stentz et al., 1989).

The C-terminus, F-R-Y, of ANP is essential for the binding of this peptide hormone to its receptor (A-type) and, hence, for its biological activity (Scarborough et al., 1989). IDE-mediated cleavage might, therefore, represent an ANP inactivation mechanism. A peptidase activity that specifically removed the C-terminal tripeptide from ANP has been demonstrated in studies on cultured vascular endothelial (Johnson et al., 1990) and smooth muscle cells (Johnson et al., 1989), and a partial characterization of this activity (Johnson & Foster, 1990) revealed enzymatic properties very similar to those of IDE. Moreover, a metalloendopeptidase that inactivates ANP by selective cleavage at the S^{25}–F^{26} bond has recently been purified from human neuroblastoma cells (Delporte et al., 1992). Like IDE, this enzyme is specifically inhibited by metal-ion chelators and sulfhydryl-modifying agents (Delporte et al., 1992). The same inhibitor profile is also characteristic of *Xenopus* proteases (Resnick et al., 1991; Carvalho et al., 1992). Analyses of the endopeptidase magaininase that splits the magainin peptides of *Xenopus laevis* revealed that the enzyme recognizes substrates on the basis of specific secondary structure rather than amino acid sequence; however, the presence of a basic residue on the carboxyl side of the cleaved peptide bond positively affects the reaction (Resnick et al., 1991). Thus, this enzyme displays similarity to IDE, and, in addition, it has the same apparent molecular mass of 110 kDa (Resnick et al., 1991). A similar mass (100 kDa) was also estimated for a second recently-described *Xenopus* peptidase (Carvalho et al., 1992). This enzyme was found to hydrolyze selected peptide bonds in several peptide hormones, including ANP at the S^{25}–F^{26} bond (Carvalho et al., 1992). The identity of the endopeptidases characterized in these studies remains unsettled. It was, however, hypothesized that they may represent prototypes of a novel family of related metalloendopeptidases (Carvalho et al., 1992). The data presented here strongly suggest that IDE is a member of this family.

The role of IDE in ANP metabolism remains to be investigated. Our data point to a preferential activity on ANP rather than the two other related natriuretic peptides. IDE may, therefore, contribute to peptide hormone inactivation in the central nervous system as well as in the circulation. Alternatively, the activity of IDE may generate new peptides with distinct physiological functions. It will be of particular interest to determine whether ANP degradation by IDE takes place intracellularly during the process of receptor-mediated endocytosis, as has been shown for insulin (Duckworth, 1988, 1990).

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