Mast cell tryptases: Examination of unusual characteristics by multiple sequence alignment and molecular modeling

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Abstract

Tryptases are trypsin-like serine proteinases found in the granules of mast cells. Although they show 40% sequence identity with trypsin and contain only 20 or 21 additional residues, tryptases display several unusual features. Unlike trypsin, the tryptases only make limited cleavages in a few proteins and are not inhibited by natural trypsin inhibitors, they form tetramers, bind heparin, and their activity on synthetic substrates is progressively inhibited as the concentration of salt increases above 0.2 M.

Unique sequence features of seven tryptases were identified by comparison to other serine proteinases. The three-dimensional structures of the tryptases were then predicted by molecular modeling based on the crystal structure of bovine trypsin. The models show two large insertions to lie on either side of the active-site cleft, suggesting an explanation for the limited activity of tryptases on protein substrates and the lack of inhibition by natural inhibitors. A group of conserved Trp residues and a unique proline-rich region make two surface hydrophobic patches that may account for the formation of tetramers and/or inhibition with increasing salt. Although they contain no consensus heparin-binding sequence, the tryptases have 10–13 more His residues than trypsin, and these are positioned on the surface of the model. In addition, clustering of Arg and Lys residues may also contribute to heparin binding. Putative Asn-linked glycosylation sites are found on the opposite side of the model from the active site. The model provides structural explanations for some of the unusual characteristics of the tryptases and a rational basis for future experiments, such as site-directed mutagenesis.

Keywords: mast cell; molecular modeling; trypsin; tryptase

Tryptases are trypsin-like serine proteinases found in mast cell secretory granules. These enzymes have received considerable attention due to postulated roles in a variety of biological and/or pathological processes, including coagulation (Maier et al., 1983; Schwartz et al., 1985; Kido & Katunuma, 1989), mast cell activation (Vassimon & Rothschild, 1990), generation of C3a from complement factor C3 (Schwartz et al., 1983), and modification of biologically active peptides (Caughey et al., 1987; Cromlish et al., 1987; Tam & Caughey, 1990). Tryptases are stored

in an active form within the cellular granules and are released along with histamine when mast cells degranulate (Schwartz et al., 1981b). Similar enzymes have been isolated and characterized from human (Schwartz et al., 1981b; Smith et al., 1984; Cromlish et al., 1987; Harvima et al., 1988a), dog (Caughey et al., 1987; Schechter et al., 1988), and rat mast cells (Kido et al., 1985). DNA cloning and sequencing has revealed the amino acid sequences of four human tryptases (Miller et al., 1989, 1990; Vanderslice et al., 1990), one dog tryptase (Vanderslice et al., 1989), and two mouse tryptases (Chu, 1990; Reynolds et al., 1990). The coding regions of these enzymes are 70% identical, but they differ by having either one or two putative glycosylation sites. The tryptases are only slightly

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larger than trypsin, with core protein molecular weights of about 27,500 due to an additional 20 or 21 amino acid residues inserted at various sites.

Although 40% identical to trypsin and chymotrypsin in amino acid sequence, the tryptases differ from other serine proteinases in several regards. Human tryptases purified from lung (Schwartz et al., 1981a; Smith et al., 1984) and skin (Harvima et al., 1988a) are stabilized by heparin, elute as a tetramer on gel filtration, only cleave specific Lys or Arg bonds in selected protein substrates, are reversibly inhibited with increasing salt concentrations above 0.2 M, and are not inhibited by natural trypsin inhibitors, such as the bovine pancreatic trypsin inhibitor (BPTI), human α -1 proteinase inhibitor, or soybean trypsin inhibitor. Similar properties have been observed for dog tryptase (Caughey et al., 1987) and rat tryptase (Kido et al., 1985), and homologous tryptases from other species probably display the same characteristics. These differences from trypsin must be due to alterations or additions to the primary sequences of tryptases, without changing the core structure that gives them the ability to cleave synthetic trypsin-substrates with Lys or Arg in the S₁ position.

In this paper we describe the predicted structure of mast cell tryptases performed by molecular modeling techniques (e.g., see review in Blundell et al., 1987) and interpret the unusual properties observed for the tryptases through identification of the unique structural features of the molecules.

Results and discussion

Heparin binding

Tryptases are packaged along with the negatively charged heparin proteoglycan in the mast cell granules, and the enzyme's binding to heparin has been used in purification schemes. In addition, heparin has been found to stabilize the activity of tryptases in low ionic strength buffers (Smith & Johnson, 1984; Schwartz & Bradford, 1986). Tryptases bind heparin in spite of their net negative charges, ranging from -4 to -10, based on the relative numbers of Asp, Glu, Lys, and Arg residues. In contrast, trypsin, which does not bind heparin, has a net positive charge of +5. The net negative charge on the tryptases is due to additional Asp and Glu residues, because tryptases have up to six more basic residues than does trypsin. Front (active site) and rear views of trypsin are shown in Figure 1A,B for comparison of the number and locations of charged residues with the tryptase model (Fig. 1C,D). Lysine and arginine side-chain amino groups are colored red, aspartic and glutamic acid side-chain oxygens are blue, and histidine imidazole rings are yellow. A number of other proteins are known to bind heparin through Lys and Arg residues, and consensus motifs, such as BBXB and BBXXB (where B is Lys or Arg), have been proposed (Blankenship et al., 1990). However, examination of the

tryptase sequences did not reveal similar heparin-binding motifs, but this was not too surprising as quite different heparin-binding sites have been reported for other proteins. For example, rat mast cell carboxypeptidase has 11 more Lys and Arg residues than the pancreatic enzyme, and these have been shown by molecular modeling to be distributed over the surface of the molecule on the side opposite to the active site (Cole et al., 1991). In the human tryptases there are several Arg and Lys residues in close proximity to each other along the left side of the tryptase model as seen in Figure 1D, and these might form a heparin-binding site.

In contrast, histidine has been shown to account for the binding of heparin to both histidine-rich glycoprotein (Burch et al., 1987) and high molecular weight kininogen (Bjork et al., 1989). The tryptases have far more histidine residues (10-13) than does trypsin with only 3. Our tryptase model shows these additional histidine residues to be positioned at or near the surface (Fig. 1C,D), whereas in trypsin only the active-site histidine is visible (Fig. 1A,B). Thus, histidines may play a role in the binding of heparin to the tryptases, but their participation would be diminished at physiological pH where histidine would be less positively charged. Human tryptase was found to bind heparin equally well at either pH 7.6 or 8.1 (Schwartz & Bradford, 1986), indicating the involvement of only highly charged residues, such as Lys and Arg. However, the pH inside the mast cell granule has been estimated to be 5.55 (De Young et al., 1987), at which the histidines would become positively charged, thus shifting the net charge of the tryptase molecules from negative to positive. Therefore, histidines probably contribute to heparin binding within the mast cell and may have a lesser role extracellularly, following degranulation.

Conserved tryptophans

A unique feature of the tryptases is the presence of nine Trp residues conserved in all tryptases, relative to only four in trypsin. Four of the tryptophans not found in trypsin (Trp-12, -14, -126, and -206) are spatially close in the tryptase models (Fig. 1F). Of all the serine proteinases, only two human plasma proteins, factor XI and plasma kallikrein, share Trp at these positions. These four Trp residues are clustered in a region on the reverse side of the model from the active site. In this region there are additional residues with hydrophobic characteristics, including Pro-13 (conserved in all serine proteinases) and Lys-11. Although Lys-11 is not unique to the tryptases, it is rare, occurring at this position only in complement factor B and two hornet chymotrypsins. Thus, only the tryptases possess this unusual combination of hydrophobic residues, which appear in the model to form a hydrophobic area available from the surface. Interestingly, these unusual residues occur as the result of mutations, rather than insertions.

Conserved prolines

A further unique feature of the tryptases relative to trypsin and all other serine proteinases is the existence of a proline-rich hydrophobic region from Leu-139 through Leu-145, with the sequence LPPPFPL. Prolines 140 and 144 are conserved in the tryptases and do not occur in other serine proteinases. Prothrombin is the only serine protease, other than the tryptases, that contains Pro at position 142. The Phe-143 residue (Tyr in mouse tryptase 1) is found only in human protein C, but protein C lacks the adjacent proline residues. This region resides along one edge of the model (colored purple in Fig. 1E), and it also seems to have arisen from mutations rather than insertions.

The formation of tetramers by the tryptases has been reported for the human, dog, and rat enzymes and is based on gel filtration data in buffers containing 1 M NaCl. Although only these enzymes have been purified and characterized, one would expect the other tryptases to form tetramers also, based on their high degree of similarity. Chromatographic characterization of the tryptases in buffers containing only physiological concentrations of salt has not been possible because the enzymes bind tightly to glass, plastics, and chromatography media (Smith et al., 1984). Additionally, the tryptases are known to be inhibited by increasing salt concentrations above 0.2 M with only 50% of their activity on benzoyl-Arg-*p*-nitroanilide in 0.2 M NaCl as compared to 0.05 M NaCl (Smith et al., 1984; Harvima et al., 1988b). This unusual characteristic has not been reported for any of the other serine proteinases. Tryptase activity is stabilized by heparin (Smith & Johnson, 1984), and this association has been proposed to contribute to tetramer formation (Schwartz & Bradford, 1986). This seems unlikely, however, because the human tryptase is dissociated from heparin at 0.8 M NaCl, which is below the concentration used in gel filtration studies. As increasing ionic strength is known to increase hydrophobic interactions, it seems more likely that the observed tetramers result from hydrophobic interactions between surface features of the monomers. The proline-rich region (residues 140–144),

which is near the end of a loop, and the tryptophan-rich pocket are possible candidates for such interactions. The proline-rich segment is positioned near the active site (Fig. 1E), where its interaction with another molecule might block the active site. Because all the tryptases have these features, homo- or heterotetramers would seem possible, but other features, such as glycosylation may also play a role. A physiological significance to the hydrophobic regions is not obvious, but they may allow the tryptases to interact with other hydrophobic structures such as membranes.

Substrate specificity and inhibition

Tryptases, although similar to trypsin in their substrate specificity with small synthetic substrates (Tanaka et al., 1983), are not highly proteolytic and have been found to make only limited cleavages in a few proteins. Recognized protein substrates for tryptases include inactivation of high molecular weight kininogen (Maier et al., 1983), inactivation of vasoactive intestinal peptide (Tam & Coughley, 1990), fibrinogen inactivation (Schwartz et al., 1985), C3a generation from complement C3 (Schwartz et al., 1983), and prothrombin activation (Kido & Katunuma, 1989). Additionally, the tryptases do not form complexes with classical trypsin inhibitors, such as alpha-1 proteinase inhibitor, BPTI, soybean trypsin inhibitor, and others (Smith et al., 1984). These observations indicate an altered substrate-binding region in the tryptases relative to trypsin. Comparison of the tryptase models with the crystal structure of the BPTI-trypsin complex showed that structural alterations around the active site might influence the binding of substrates and inhibitors. A loop, containing nine inserted residues in the region from 164 through 174 (colored deep red in Fig. 1E), lies adjacent to the active-site cleft. The fold of this large insert could differ from the model, but its position relative to the active site strongly suggests that it influences substrate specificity and/or complexation with inhibitors. Another insertion involves residues 20–26 (colored orange in Fig. 1E), also located near the active site where they too may influence substrate and/or inhibitor binding.

Fig. 1. Space-filling models showing the X-ray structure of trypsin (**A** and **B**) and predicted structure of human skin tryptase 1 (**C–F**). **A**, **C**, and **E** are of the active site face, whereas **B**, **D**, and **F** are of the side opposite the active site. In **A**, **C**, and **E**, the active site Ser is colored green and all His residues are yellow including the active site His adjacent to the active site Ser at the center of the frames. In **A–D**, Asp and Glu side-chain carboxyl oxygen atoms are colored blue, whereas Arg and Lys residue side-chain nitrogens are red.

The charge distribution on the active-site face of trypsin is shown in **A**. In addition, those regions where major insertions occur in tryptase are highlighted for comparison of size and position with **E**. The Ser, Gly, and Tyr residues, where a large insertion occurs in tryptase from residues 20 through 26, are colored orange, whereas the Gly and Gln residues at 169–170 (see Fig. 3 for numbering) are shown in deep red. The distribution of charged residues on the reverse side of trypsin in which no His residues are visible is illustrated in **B**. The distribution of charged residues on the active-site face and reverse sides of tryptase, respectively, are shown in **C** and **D**. Major insertions in tryptase relative to trypsin, with the 20–26-residue insertion colored orange and the 164–174 insertion colored deep red, are shown in **E**. Additionally, the proline-rich hydrophobic region of tryptase is colored purple in this frame. The tryptophan-rich hydrophobic region on the reverse side of the tryptase model is colored purple, and the nitrogens of the putative Asn-linked glycosylation sites are colored pale blue/green (**F**).

Fig. 2. C-alpha trace stereo view of the tryptase model, viewed down the active-site cleft. Every tenth residue is labeled with the residue type and number. All atoms of the active site His⁴⁴, Asp⁹¹, and Ser¹⁹⁴ residues are drawn with thick lines.

Although these changes are difficult to interpret due to uncertainties in the modeling, they do offer the first plausible explanation for the high degree of specificity displayed by the tryptases. Additionally, a stereo-pairs diagram of the model is displayed in Figure 2 to show general folding and the positioning of inserted loops.

Asn-linked glycosylations

All the human tryptases have a putative Asn-linked glycosylation site at residue 203, which is positioned adjacent to the region of conserved tryptophans (colored light blue in Fig. 1F). One would speculate that glycosylation at this site may serve to shield the hydrophobic tryptophan-rich area from the water or prevent interactions with other hydrophobic molecules. Another potential glycosylation site occurs near the top edge, but to the rear and away from the active site. It is present in both mouse tryptases, dog tryptase, human lung tryptase α , and human skin tryptase 1. Only mouse tryptase 2 has a glycosylation site at residue 21, which is on the active-site face of the enzyme model. It is difficult to speculate concerning possible functional roles for these glycosylation sites. Yet, the different spatial positions of the glycosylations within this group of enzymes from the same cell type suggests that glycosylation may serve to alter function and/or targeting of the different tryptases.

Potential uses of the models

The mast cell tryptases were good candidates for molecular modeling. Their homology with trypsin and position within the serine proteinase family facilitated an examination of their structural features to offer explanations for their unique and unusual functional characteristics. Although the models may lack the accuracy needed for

a careful examination of substrate or inhibitor interactions, they provide a logical starting point for detailed investigations of the structural features defining the novel properties of the mast cell tryptases. Site-directed mutagenesis of unique regions in the tryptases relative to those of trypsin would be one method of testing the hypotheses developed from this modeling exercise. For example, converting the large insertions in the region from 164 through 174 back to the sequence of trypsin might reduce specificity with regard to protein substrates. Likewise mutation of the unique proline-rich region to the corresponding residues in trypsin could be used to test the hypothesis that these are involved in tetramer formation. Similar experiments could be performed with other residues unique to the tryptases to test their roles in heparin binding and other functions. Some of these questions may be answered by the purification or expression of the individual enzymes, because at present the purified enzyme preparations have not been correlated with specific gene products and amino acid sequences. Thus, our model provides a basis for the rational design of experiments to study the relationships of structure to function in the mast cell tryptases.

Materials and methods

Sequence alignment

The tryptase sequences were multiply aligned with trypsin, chymotrypsin, and several members of the trypsin family of enzymes, using the AMPS package (Barton & Sternberg, 1987; Barton, 1990). The high level of similarity between the tryptases and other serine proteinases gave a largely unambiguous multiple sequence alignment. The positions of insertions and deletions in the alignment were scrutinized in relation to the three-dimensional

structures of trypsin and chymotrypsin and small adjustments made to the alignment to avoid putting insertions, relative to trypsin, in regions of defined secondary structure. One exception was in the region 201–209, where insertions extended into a β -strand. However, similar insertions occur in chymotrypsin in this region and the available crystal structure was used to guide the modeling of these insertions. The final alignment used for the construction of the models is shown in Figure 3, with numbering from 1 to 245 based on the tryptases. In this numbering system the active-site residues corresponding to Ser-195, His-57, and Asp-102 in the chymotrypsinogen numbering system are Ser-194, His-44, and Asp-91. Likewise Asp-189, which contributes to trypsin's specificity for cleavage at Lys and Arg residues due to its position at the bottom of the S_1 substrate-binding pocket, is Asp-

188 in the tryptase sequences. Only human lung tryptase- α differs from the others by having a single deletion at position 60, relative to the other tryptases. Although models of several of the tryptases were constructed, no striking differences were observed between them, except for glycosylation positions. Accordingly, because the human tryptases are the best characterized members of the group, we limit our presentation to the human skin tryptase 1 model.

Modeling

The molecular modeling program Quanta 3.0 running on a Silicon Graphics Personal Iris workstation was used to construct the tryptase models. The homology modeling functions of Quanta were first used to construct a model

Fig. 3. Sequence alignment used for modeling the mast cell tryptases showing the sequences of the tryptases in comparison to those of trypsin and chymotrypsin. HST1, human skin tryptase 1; HST2/HLT β , human skin tryptase 2/human lung tryptase beta; HST3, human skin tryptase 3; HLT α , human lung tryptase alpha; MMCT1, mouse mast cell tryptase 1; MMCT2, mouse mast cell tryptase 2; DTRYPT, dog mast cell tryptase; 2ptn, bovine trypsin; 2cga, bovine chymotrypsin. The secondary structure of trypsin and chymotrypsin as defined by the program DSSP (Kabsch & Sander, 1983) is shown by the lines 2ptn-SS and 2cga-SS, where H denotes α -helix, G is 3_{10} -helix, E is β -strand, B is an isolated β -bridge, T is a hydrogen-bonded turn, and S is a bend structure (for details of these definitions see Kabsch & Sander [1983]). Numbering follows HST1, whereas asterisks, *, above and below the alignment highlight the active-site His, Asp, and Ser residues. Exclamation marks, !, highlight the Asp in the S_1 -binding pocket. Asn-linked glycosylation sites are shown in lowercase italicized bold print.

of mouse mast cell tryptase 2 based on the crystal structure of bovine trypsin (Marquart et al., 1982). Briefly, the alignment shown in Figure 3 was used to guide the substitution of side-chain types in regions not aligned with gaps, followed by the formation of the four highly conserved disulfides. Insertions were then regularized to give reasonable geometry (two cycles of 100 steps of Steepest Descents energy minimization was performed over the inserted sequence, including two residues on either end of the modeled region). The largest insertion, involving 9 added residues in an 11-residue region over positions 164–174, was the most difficult segment to model, and was interactively folded into an acceptable conformation. The model was then analyzed for unfavorable Van der Waals contacts, which were relieved by adjusting the appropriate side chain torsion angles. The active-site Ser, His, and Asp residues, as well as the Asp in the S_1 -binding pocket were constrained prior to energy minimization with CHARMm. The minimized average energy of the model was negative and of a similar magnitude to that calculated for the crystal structure of trypsin. The resulting model was subsequently used as the scaffold for construction of additional tryptase models via the Quanta homology modeling package.

Model interpretation

The tryptases were examined for unique and unusual regions, conserved among the tryptases but different from other serine proteinases, by aligning them with 100 closely related proteins chosen by searching the NBRF-PIR data bank (version 28) with the sequence of mouse mast cell tryptase 1, using the Smith–Waterman dynamic programming algorithm (Smith & Waterman, 1981) (program sw, G.J.B., unpubl.) with Dayhoff's xMDM78 matrix (Dayhoff et al., 1978) and a gap penalty of 8. The aligned sequences included several chymases, which are mast cell serine proteinases with chymotrypsin-like activity, to look for unique sequences common to both of these mast cell serine proteinases that might aid in relating structural features to the unusual characteristics of the tryptases. If, for example, a common hydrophobic region had been found, its role in tetramer formation could have been discounted, because the chymases do not form tetramers.

Comparison of the models was performed by superimposing them with the program SCAMP (Barton & Sternberg, 1988) running on an Evans and Sutherland PS390 terminal. Structural features unique to the tryptases were observed by coloring insertions and unusual mutations differently while superimposing the models on the structure of trypsin.

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