Formation of active tryptase monomers in the presence of low molecular weight heparin

Mast cell tryptase is stored as an active tetramer in complex with heparin in mast cell secretory granules. Previously, we demonstrated the dependence on heparin for the activation/tetramer formation of a recombinant tryptase. Here we have investigated the structural requirements for this activation process. The ability of heparin-related saccharides to activate a recombinant murine tryptase, mouse mast cell protease-6 (mMCP-6), was strongly dependent on anionic charge density and size. The dose-response curve for heparin-induced mMCP-6 activation displayed a bell-shaped appearance, indicating that heparin acts by binding to more than one tryptase monomer simultaneously. The minimal heparin oligosaccharide required for binding to mMCP-6 was 8–10 saccharide units. Gel filtration analyses showed that such short oligosaccharides were unable to generate tryptase tetramers, but instead gave rise to active mMCP-6 monomers. The active monomers were inhibited by bovine pancreatic trypsin inhibitor, whereas the tetramers were resistant. Furthermore, monomeric (but not tetrameric) mMCP-6 degraded fibrinogen. Our results suggest a model for tryptase tetramer formation that involves bridging of tryptase monomers by heparin or other highly sulfated polysaccharides of sufficient chain length. Moreover, our results raise the possibility that some of the reported activities of tryptase may be related to active tryptase monomers that may be formed according to the mechanism described here.

Received for publication, June 15, 2001, and in revised form, August 6, 2001
Published, JBC Papers in Press, August 31, 2001, DOI 10.1074/jbc.M105831200

Jenny Hallgren‡, Dorothe Spillmann§, and Gunnar Pejler¶†
From the ‡Department of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences, and the ¶Department of Medical Biochemistry and Microbiology, Uppsala University, S-751 23 Uppsala, Sweden

© 2001 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.
Mechanism for Tryptase Activation

42775

ization (10). A recombinant mouse tryptase, mouse mast cell protease-6 (mMCP-6),1 was expressed with an N-terminal His tag followed by an enterokinase cleavage site replacing the native activation peptide. Enterokinase cleavage of this fusion protein thus yielded the mature tryptase monomer. Using this recombinant tryptase, we showed that heparin is required for the assembly of monomeric inactive tryptase to active tetramers and that tetramerization only occurs at acidic pH (below ~6.5) (10). The strong pH dependence of the tetramerization process indicated that histidine residues, which are positively charged at pH <6.5, but uncharged at neutral pH, may be involved in the interaction with heparin.

In this study, we have investigated the mechanism and structural requirements (in terms of heparin) for the assembly of inactive tryptase monomers into active tetramers. Our results indicate that tryptase tetramerization involves bridging of tryptase monomers by heparin or similar highly sulfated polysaccharides of sufficient chain length. When heparin oligosaccharides of shorter length are used, active tryptase monomers are instead formed.

EXPERIMENTAL PROCEDURES

All studies were performed using mMCP-6. The expression, purification, and activation of mMCP-6 have been previously described (10). Briefly, a mMCP-6 construct inserted in the pCEP-Pu2 vector was transfected into human 293 cells. This vector also contained the BM40 signal peptide, which ensures secretion of the protein into the culture medium. The recombinant protein was constructed with an N-terminal 6-histidine tag followed by an enterokinase (EK) cleavage site, which replaced the natural activation peptide. His6-EK-mMCP-6 was purified on Ni2+-nitrilotriacetic acid-agarose (QIAGEN GmbH, Hilden, Germany) according to instructions provided by the manufacturer. To obtain mature mMCP-6 monomers, the His6-EK-mMCP-6 fusion protein was digested overnight (37°C) with enterokinase at an enterokinase/mMCP-6 ratio of 1:500 (w/w) in PBS (10 mM phosphate, 0.14 mM NaCl, and 2.7 mM KCl, pH 6). Active mMCP-6 was obtained after incubating the protein with heparin at pH 6 as described (10) (see also below). Unlabeled pig mucosal heparin (Mn ~15,000), heparan sulfate from whale lung, dermatan sulfate, and 3H-labeled heparin (25,000 cpm/µg) were gifts from Ulf Lindahl (Uppsala University, Uppsala, Sweden). Heparin proteoglycan (Mn ~1 x 106) was purified from rat peritoneal mast cells as described previously (26). Chondroitin sulfates A, C, and E were purchased from Calbiochem (Darmstadt, Germany). Dextran sulfate (Mw ~8000) was obtained from Sigma. Unlabeled and 3H-labeled heparin oligosaccharides (5000 cpn/ml) and the releasable dimeric tryptase preparations (N-desulfated/N-acetylated heparin, 2-O-desulfated/2-sulfated, 2-O-desulfated/2-desulfated, heparin, and 6,0-desulfated heparin) were as described (27–29). Enterokinase was from Roche Molecular Biochemicals (Mannheim, Germany). The chromogenic substrate S-2288 (H-β-Ile-Pro-Arg-p-nitroanilide) was from Chromogenix (Mölndal, Sweden). Bovine pancreatic trypsin inhibitor (BPTI) was purchased from Sigma. SDS-polyacrylamide gel electrophoresis in a Laemmli system was performed with 12% polyacrylamide gels under nonreducing conditions. Gels were stained with Coomassie Blue.

Enzymatic Assays—Enzymatic assays were performed in 96-well microtiter plates. mMCP-6 (100–200 ng) was incubated with different saccharides (445 pmol to 44.5 µmol) in PBS (total volume of 130–380 µl), pH 6, for 30 min. In one set of experiments, the standard PBS buffer (0.14 mM NaCl) was replaced by phosphate buffers containing 0–0.5 mM NaCl. Subsequently, mMCP-6 activity was recorded using the chromogenic substrate S-2288 for tryptase activity after addition of 20 µl of 2 mM S-2288 in H2O. Binding Assays—The binding of mMCP-6 to heparin was studied using the nitrocellulose filter disc assay of Pejler (30) with modifications. 3 µg of enterokinase-digested His6-EK-mMCP-6 was incubated with [3H]heparin (intact; 0.48–360 pmol, 180–136,000 cpm) or [1H]heparin oligosaccharides of various sizes (80 pmol; 4500 cpm) in a total volume of 400 µl of PBS, pH 6, for 30 min. The incubation mixtures were rapidly passed through prewashed (with PBS, pH 6) nitrocellulose filters (25-mm diameter, 0.45-µm pore size; Sartorius Corp.) using a vacuum suction apparatus. Subsequently, the filters were washed with PBS, pH 6 (2 x 5 ml), and transferred into scintillation vials. Bound radioactivity was eluted from the filters by addition of 2 ml of 100 mM NaOH and 2 mM NaCl. After 30 min, 5 ml of scintillation fluid (OptiPhase HiPhase 3, Wallac) was added, and radioactivity was quantified with a β-scintillation counter.

Size-exclusion Gel Chromatography—Size-exclusion gel chromatography was performed in a fast protein liquid chromatography system (Amersham Pharmacia Biotech) using a Superdex 200 column (10 x 300 mm). The column was equilibrated and eluted with PBS, pH 6, and run at a flow rate of 0.5 ml/min. Calibration was performed with the following standards (all from Sigma): carbonic anhydrase (29 kDa), bovine serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), α-amylase (200 kDa), and blue dextran (2000 kDa). Enterokinase-digested His6-EK-mMCP-6 (10–20 µg, 0.33–0.66 nmol) was analyzed either alone or after preincubation with 3.3 nmol of either intact heparin or various heparin oligosaccharides of 20, 10, or 4 saccharide units. Absorbance was recorded at 280 nm. 0.5-ml fractions were collected from which 200-µl samples were analyzed for tryptase activity after addition of 20 µl of 2 mM S-2288. In the inhibition studies, 100 µl of the fractions was transferred to 96-well plates and either not treated further or mixed with 2 µg of protease inhibitor (BPTI, α1-antitrypsin, soybean trypsin inhibitor) in 10 µl of PBS. After an incubation time of 30 min, residual tryptase activity was determined as described above.

RESULTS

Structural Requirements for the Activation of mMCP-6 by Heparin—In a previous study, we showed that the activation and tetramer formation of a recombinant tryptase, mMCP-6, is dependent on heparin and acidic pH (10). In this study, we examined the mechanism and structural requirements for the heparin-induced tryptase activation. First, we investigated whether other structurally related saccharides were capable of replacing heparin as tryptase activators. Monomeric mMCP-6 was incubated with heparin, heparin proteoglycan, heparan sulfate, dermatan sulfate, chondroitin sulfate (A, C, or E), or dextran sulfate at increasing concentrations and was then assayed with the chromogenic substrate S-2288 for tryptase activity (Fig. 1). As expected, both heparin and heparin proteoglycan produced active tryptase. In addition, dextran sulfate proved to be an effective tryptase activator. In fact, dextran sulfate was even more effective than heparin. In contrast, heparan sulfate, dermatan sulfate, and chondroitin sulfates A and C did not give any detectable activation of mMCP-6. Chondroitin sulfate E at high concentrations produced measurable

---

1 The abbreviations used are: mMCP-6, mouse mast cell protease-6; EK, enterokinase; PBS, phosphate-buffered saline; BPTI, bovine pancreatic trypsin inhibitor.
trypsinase activation, although the levels of activity were much lower than those obtained with heparin or dextran sulfate.

To investigate the heparin size requirements for mMCP-6 activation, we used heparin oligosaccharides of increasing chain lengths (4–26 saccharide units). None of these oligosaccharides were as effective as intact heparin in production of active trypsinase (Fig. 2). However, clearly measurable levels of trypsinase activity were seen after incubation with several of the various oligosaccharides, and an increased ability to activate mMCP-6 with increasing chain length was observed.

A possible explanation for the limited ability of the heparin oligosaccharides to produce active trypsinase could be that shorter heparin chains require longer incubation times to achieve trypsinase activation. To test this, we compared heparin and 20-unit saccharides at three different concentrations for their ability to activate mMCP-6 over time. The results shown in Fig. 3 demonstrate that the 20-unit saccharides gave a lower level of maximal activity compared with intact heparin, in agreement with the results shown in Fig. 2. Furthermore, the maximal levels of activities were reached at equal rates both for the 20-unit saccharides and intact heparin.

Heparin contains sulfate groups at various positions: the uronic acid residues (both GlcUA and IdoUA) can be O-sulfated, and the glucosamine residues can carry 6-O-sulfate and/or N-sulfate groups. In addition, in rare cases, the glucosamine residues may contain 3-O-sulfate groups. To determine whether trypsinase activation shows a requirement for sulfate groups located at any of these specific positions, we tested whether tryptase activation requires sulfate groups at low NaCl concentrations, up to ~0.14 M (Fig. 4). At higher NaCl concentrations, the possibility to obtain active trypsinase after addition of heparin fell drastically, with complete inhibition of the activation process at NaCl concentrations above ~0.3 M (Fig. 5).

**Mechanism for Tryptase Activation**

To investigate if tryptase activation is dependent on the concentrations of trypsinase and heparin, three concentrations of mMCP-6 were incubated with increasing concentrations of heparin (Fig. 6). The results show that the specific activity of mMCP-6 increased markedly with increasing mMCP-6 concentration. Furthermore, at all mMCP-6 concentrations, we noted bell-shaped dose-response curves for heparin, where the trypsinase activity levels first reached a maximal

![Figure 2](image2.png)

**Fig. 2.** Dependence on heparin size for mMCP-6 activation. 100 ng of enterokinase-digested His<sub>6</sub>-EK-mMCP-6 (25 nM) was incubated with different heparin oligosaccharides (~4.5 μM to 4.5 μM; 4-unit saccharide (open squares), 6-unit saccharide (closed squares), 8-unit saccharide (stippled squares), 10-unit saccharide (open diamonds), 12-unit saccharide (closed diamonds), 14-unit saccharide (stippled diamonds), 16-unit saccharide (open circles), 18-unit saccharide (closed circles), 20-unit saccharide (stippled circles), 22-unit saccharide (open triangles), 24-unit saccharide (closed triangles), 26-unit saccharide (stippled triangles)) or intact heparin (×) in a total volume of 130 μl of PBS, pH 6. After 30 min, 20 μl of 2 mM S-2288 was added, followed by measurement of trypsinase activity. The results shown represent the means ± S.D. of triplicate determinations (error bars are frequently hidden by the symbols).

![Figure 3](image3.png)

**Fig. 3.** Time course for the activation of mMCP-6 by intact heparin and heparin 20-unit saccharides. 2.3 μg of enterokinase-digested His<sub>6</sub>-EK-mMCP-6 (590 nM) was incubated in the presence of ~44.5 mM (squares), ~445 nM (circles), or ~4.45 μM (triangles) 20-unit saccharide (closed symbols) or intact heparin (open symbols) in a total volume of 130 μl of PBS, pH 6. After various periods of time, 20 μl aliquots of the incubation mixtures were transferred to 96-well plates and mixed with 100 μl of PBS, pH 6. Finally, 20 μl of 2 mM S-2288 was added, followed by measurement of trypsinase activity. The results shown represent the means ± S.D. of triplicate determinations (error bars are frequently hidden by the symbols).

![Figure 4](image4.png)

**Fig. 4.** Activation of mMCP-6 by selectively desulfated heparin derivatives. 100 ng of enterokinase-digested His<sub>6</sub>-EK-mMCP-6 (25 nM) was incubated with different amounts (6.7 ng/ml to 67 μg/ml, ~0.61 mM to 6.1 μM) of various heparins (unmodified heparin (■), N-desulfated/N-acetylated heparin (△), 2-O-desulfated/N-sulfated heparin (●), 2-O-desulfated heparin (○), and 6-O-desulfated heparin (▲)) in a total volume of 130 μl of PBS, pH 6.0. After 30 min, 20 μl of 2 mM S-2288 was added, followed by measurement of trypsinase activity. The results shown represent the means ± S.D. of triplicate determinations (error bars are frequently hidden by the symbols).
plateau, but then decreased markedly at excess heparin concentrations (Fig. 6).

**Binding of mMCP-6 to Heparin**—Binding studies were performed in which monomeric mMCP-6 was incubated with 3H-labeled heparin. Tryptase-bound radioactivity was then determined using the nitrocellulose filter disc assay (see “Experimental Procedures”). The same samples were also assayed for tryptase activity to directly correlate binding and activation (Fig. 7). Saturation of the binding occurred at a heparin/trypase ratio of ~1:1. However, the activity continued to increase at higher heparin concentrations than those required for maximal binding. Next, binding studies were performed in which 3H-labeled oligosaccharides (4–24 saccharide units) were incubated with mMCP-6, followed by the nitrocellulose filter disc assay. The results shown in Fig. 8 indicate that the minimal heparin chain size required for binding was 8–10 saccharide units.

**Formation of Active Tryptase Monomers**—In a previous report, we showed that addition of intact heparin to mMCP-6 monomers results in the formation of active tetramers (10). Here we used size-exclusion gel chromatography to study the effect of heparin oligosaccharides on mMCP-6 tetramerization. Mature monomeric mMCP-6 was incubated either alone or together with heparin 4-, 10-, or 20-unit saccharides or intact heparin and analyzed on a Superdex 200 column (Fig. 9). In the absence of added heparin, no tetramer formation was seen, and no enzymatic activity was detected (Fig. 9A). Addition of intact heparin transferred approximately half of the material into tetramers and resulted in generation of enzymatic activity, of which essentially all was found in the tetramer fraction (Fig. 9B). Previous studies showed that if heparin is added at a larger molar excess, complete tetramerization is obtained (10). Incubation of monomeric mMCP-6 with heparin 4-unit saccharides did not result in any detectable tetramerization or activation (Fig. 9C). No tetramerization was obtained with the 10-unit saccharide. However, clearly detectable activity was found in the monomer peak after addition of heparin 10-unit saccharides to mMCP-6 (Fig. 9D). Incubation of monomeric mMCP-6 with 20-unit saccharides produced a detectable tetramer peak (~15% of the protein), although the majority of the protein eluted in the monomer fraction. Again, the monomer fractions displayed enzymatic activity. However, comparison of the height of the protein (A$_{280}$) and activity peaks indicated...
that the specific activity was considerably higher for the tryptase recovered in the tetramer fraction compared with the monomer fraction. To make sure that the activity found in the monomer fraction was due to monomers and not tetramers that had been assembled after the chromatography step, monomer fractions were pooled and rerun on the Superdex 200 column. Again, the activity was found in the monomer position (data not shown).

To obtain further proof for the formation of active monomers, we compared the susceptibility of the tetramer and monomer fractions of mMCP-6 to various protease inhibitors. Neither soybean trypsin inhibitor nor α1-antitrypsin inhibited tryptase activities eluted in the tetramer or monomer fractions (data not shown). In contrast, BPTI inhibited the tryptase activity in the monomer (but not tetramer) fractions (Fig. 10). Both the monomeric and tetrameric tryptases were inhibited by protamine, a polycationic heparin antagonist (data not shown).

Earlier studies have indicated that fibronectin is a substrate for tryptase (33, 34). Further experiments were conducted to assess whether fibronectin is a substrate for both tetrameric and monomeric tryptases. Monomeric active tryptase was obtained after incubation of monomeric mMCP-6 with 20-unit saccharides, followed by separation of active tetramers and monomers by size-exclusion gel chromatography (Fig. 9). The results shown in Fig. 11 demonstrate that fibronectin was degraded by monomeric mMCP-6, but not by mMCP-6 in tetrameric form.

**DISCUSSION**

This study was undertaken to investigate the mechanism and structural requirements in the heparin-induced activation/tetramerization of tryptase. Mature mouse tryptase monomers were obtained by enterokinase digestion of a fusion protein containing mature monomeric mMCP-6 expressed with an N-terminal His6 tag followed by an enterokinase site replacing the natural activation peptide. The structural requirements in the activation of tryptase were studied by comparing the potency of heparin and various other structurally related compounds. Both intact heparin chains and heparin in its proteoglycan form were efficient activators. In contrast, heparan sulfate, dermatan sulfate, and chondroitin sulfates (A, C, and E) were not efficient tryptase activators. Since these compounds are all less sulfated than heparin, our results indicate

**FIG. 9. Effects of heparin oligosaccharides on macromolecular organization of mMCP-6.** Enterokinase-digested His6-EK-mMCP-6 (10 μg, 1.67 μM) was incubated alone (A) or in the presence of 16.5 μM intact heparin (B), 4-unit saccharide (C), 10-unit saccharide (D), or 20-unit saccharide (E) in a total volume of 200 μl of PBS, pH 6. After 30 min of incubation, the samples were analyzed on a Superdex 200 column eluted with PBS, pH 6. Absorbance at 280 nm (---) was recorded. Fractions (0.5 ml) were collected and assayed for tryptase activity with S-2288 (○).

**FIG. 10. Inhibition of tryptase monomer activity by BPTI.** Enterokinase-digested His6-EK-mMCP-6 (20 μg, 3.3 μM) was incubated with 30 μg of heparin 20-unit saccharide (29 μM) in a total volume of 200 μl of PBS, pH 6. After 30 min, the sample was subjected to size-exclusion gel chromatography on a Superdex 200 column. Fractions (0.5 ml) were collected and assayed for tryptase activity in the absence (□) or presence (■) of BPTI.
Mechanism for Tryptase Activation

**Fig. 11. Degradation of fibronectin by mMCP-6 monomers.** Enterokinase-digested His₆-EK-mMCP-6 (17.4 μg, 1.2 μM) was incubated with 30 μg of heparin 20-unit saccharide (11.6 μM) in a total volume of 500 μl of PBS, pH 6, followed by separation of tryptase tetramers and monomers (see Fig. 9E). Fractions containing the highest tryptase activities were pooled, and 100 μl of these samples was mixed with 60 μg of fibronectin and incubated at 25 °C. As a control, fibronectin was incubated in the absence of tryptase. Samples (25 μl) from the tryptase/fibronectin incubation mixtures were removed after 1, 4, and 20 h and subjected to SDS-polyacrylamide gel electrophoresis under nonreducing conditions.

that efficient tryptase activation requires binding of tryptase to highly sulfated compounds. It is interesting to note that of these latter polysaccharides, only chondroitin sulfate E gave any detectable activation of mMCP-6. Chondroitin sulfate E has a higher sulfate content than both chondroitin sulfates A and C, in agreement with a dependence of tryptase activation on the anionic character of its ligand. Dextran sulfate, a synthetic polysaccharide with a high sulfate content, activated tryptase with an even higher efficiency than heparin. Since dextran sulfate has a carbohydrate backbone structure that differs drastically from that of heparin, this finding further supports that the major factor for tryptase activation is anionic charge density rather than more specific structural requirements. This was also supported by experiments with structurally modified heparins in which the N-sulfate, 2-O-sulfate, or 6-O-sulfate groups had been selectively removed. All of these modified heparins were approximately equally effective tryptase activators, suggesting that the interaction of tryptase with heparin is not dependent on sulfate groups at any particular position.

Heparin oligosaccharides composed of up to 26 monosaccharides were much less efficient in producing active tryptase than was intact heparin, indicating that tryptase activation is strongly size-dependent regarding heparin chain length. Studies were undertaken to investigate if the ability to activate tryptase is a direct function of tryptase-binding properties. Such experiments showed that oligosaccharides (e.g. 8–10 saccharide units) that were relatively inefficient in producing tryptase activation could bind to tryptase. This raises the possibility that tryptase binding does not necessarily lead to tetramerization and activation. A possible explanation would be that tetramerization requires that at least two monomers are bound alongside the same heparin molecule, whereby heparin thus “bridges” these monomers to facilitate intermolecular contact (Fig. 12). This would explain why heparin oligosaccharides of sufficient size for binding (e.g. 10 saccharide units) are relatively unable to cause efficient tryptase tetramerization. According to such a mechanism, bridging would require that at least two tryptase monomers interact with the same heparin molecule, i.e. forming a ternary complex. If this is the case, tryptase activation would be expected to be most efficient at heparin concentrations that are optimal for promoting formation of ternary complexes between heparin and at least two tryptase monomers. At higher heparin concentrations, there should be a reduced probability that monomers are bound to the same heparin chain since the excess of heparin should favor formation of binary complexes between heparin and solitary tryptase monomers. Indeed, our results illustrate that tryptase activation shows a bell-shaped dose-response curve, with reduced tryptase activation at excess heparin concentrations, in agreement with such a mechanism. It should be noted that similar bell-shaped dose-response curves have been observed in other processes in which heparin acts by bridging two heparin-binding protein molecules, e.g. the acceleration of antithrombin-mediated thrombin inactivation (35) and chymase-catalyzed thrombin degradation (36).

When we compared the capability of heparin to bind to and activate tryptase, we observed that, although the binding was saturated at an ~1:1 molar ratio of heparin to tryptase monomer, tryptase activity continued to increase at higher heparin concentrations. In fact, optimal tryptase activation was seen at a molar ratio of ~200:1. The explanation for this apparent discrepancy is not certain. One possibility could be that tryptase contains both high and low affinity binding sites for heparin and, if this is the case, that optimal tryptase activation requires binding of heparin to both high and low affinity binding sites. Since the nitrocellulose filter disc assay we used for studying binding of heparin to tryptase involves a washing step, this assay represents a non-equilibrium system. The assay is therefore likely to detect high affinity interactions, whereas loosely bound heparin molecules may detach during the washing step and thus escape detection. In contrast, the equilibrium between heparin and tryptase is not disturbed in the activity assay. This assay should therefore allow detection of both high and low affinity interactions.

It is well established that high concentrations of NaCl stabilize human tryptase (22, 32). A likely interpretation for this observation would be that hydrophobic interactions are important in tryptase tetramer stabilization. However, we have shown here that there is an opposite relation between NaCl concentration and ability to form active tryptase. Optimal tryptase activation occurred at NaCl concentrations up to ~0.14 M, whereas tryptase activation was completely abrogated at NaCl concentrations above ~0.3 M. Since NaCl would interfere with electrostatic interactions, but strengthen hydrophobic interactions, we conclude that electrostatic interactions are of higher importance than the hydrophobic counterparts during tryptase activation, at least during the initial phases of tetramer assembly. Once heparin has bound to tryptase, it is quite likely that additional hydrophobic interactions between different monomers contribute to stabilizing the tetramer structure, as indicated from the crystal structure of human tryptase (6).

We have shown here, using size-exclusion gel chromatography, that short heparin oligosaccharides (e.g. 10 saccharide units) are capable of producing active tryptase monomers rather than tetramers. Further proof that the compounds present in the monomer position actually corresponded to active monomers (and not to tetramers that had been assembled after the chromatography step) came from inhibition studies in which BPTI was shown to inhibit tryptase in the monomer (but not tetramer) fraction. Resistance to macromolecular protease inhibitors is a classical feature of the mast cell tryptase tetramer (9) and is explained by its molecular organization, where the active sites that are located in the central pore are inaccessible to large protease inhibitors due to steric reasons (6, 7).

The access to the active site in an active monomer would obviously be less restricted, which would facilitate interaction with macromolecular protease inhibitors. The inhibition of the tryptase activity that was eluted from the Superdex 200 column in the position of monomers (but not tetramers) thus provides strong evidence that the active material eluting in the monomer fraction corresponds to true active tryptase monomers.

Tryptase has been reported to degrade several large pro-
The intact heparin allows binding of more than one tryptase monomer to the same heparin molecule (steps 1 and 2). If two tryptase monomers are bound adjacent (step 3), monomer-monomer interaction may be initiated along with subsequent tetramer assembly (step 4). In the model depicted, two heparin chains are bound to each tryptase tetramer. However, we cannot rule out that one heparin chain can accommodate all four subunits of the tetramer.

Interaction of tryptase monomers with heparin oligosaccharides that are not sufficiently long to accommodate two monomers may result in the formation of active monomers.

When heparin is dissociated from the active tryptase monomer, activation of this monomer is not clear, but may involve conformational changes that are induced upon heparin binding. When heparin is dissociated from the active tryptase monomer, its conformation can be distorted, which may lead to loss of enzymatic activity.

The formation of active monomers in the presence of short heparin oligosaccharides clearly supports the suggested mechanism for heparin-induced tryptase activation/tetramerization (Fig. 12). Examination of the crystal structure for human β-trypase revealed that a positively charged patch is present along two neighboring tryptase units in the tetramer and that this patch may accommodate a heparin chain of ~20 monosaccharide units in length (6). Possibly, this chain length corresponds to the minimal size required for the bridging of two tryptase monomers and thus for tetramerization. Accordingly, an oligosaccharide chain length of ~10 monosaccharide units would be expected to be required for interaction with each tryptase monomer. Oligosaccharides of this size should consequently be able to interact only with one tryptase molecule and would therefore be capable of producing active monomers only (Fig. 12). Our results indicate that 8–10 saccharide units indeed represent the minimal oligosaccharides required for tryptase binding, in close agreement with such a notion. Furthermore, our data indeed indicate that these smaller oligosaccharides are relatively unable to cause tetramerization, but instead preferentially promote formation of active monomers. In summary, we suggest a model for the formation of active tryptase tetramers in which tryptase monomers are bridged by heparin, or similar highly charged polysaccharides, of sufficient chain length (Fig. 12).

Acknowledgment—We are grateful to Ingemar Björk for critical reading of the manuscript.
26. Pejler, G., Soderstrom, K., and Karlstrom, A. (1994) Biochem. J. 299, 507–513
27. Feyzi, E., Lustig, F., Fager, G., Spillmann, D., Lindahl, U., and Salmivirta, M. (1997) J. Biol. Chem. 272, 5518–5524
28. Feyzi, E., Trybala, E., Bergstrom, T., Lindahl, U., and Spillmann, D. (1997) J. Biol. Chem. 272, 24850–24857
29. Pejler, G., Lindahl, U., Larm, O., Scholander, E., Sandgren, E., and Lundblad, A. (1988) J. Biol. Chem. 263, 5197–5201
30. Pejler, G. (1996) Biochem. J. 320, 897–903
31. Kozik, A., Potempa, J., and Travis, J. (1998) Biochim. Biophys. Acta 1385, 139–148
32. Alter, S. C., Metcalfe, D. D., Bradford, T. R., and Schwartz, L. B. (1987) Biochem. J. 248, 821–827
33. Lehi, J., Harvima, I., and Keski-Oja, J. (1992) J. Cell. Biochem. 50, 337–349
34. Kaminaka, K., Helisalmi, P., Harvima, R. J., Naahikainen, A., Horismanheimo, M., and Harvima, I. T. (1999) J. Invest. Dermatol. 113, 567–573
35. Jordan, R. E., Oosta, G. M., Gardner, W. T., and Rosenberg, R. D. (1986) J. Biol. Chem. 255, 10081–10090
36. Pejler, G., and Sadler, J. E. (1999) Biochemistry 38, 12187–12195
37. Kelty, C. M., Lees, M., Shuttleworth, C. A., and Woolley, D. (1993) Biochem. Biophys. Res. Commun. 191, 1230–1236