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Regulation of the activity of human chymase during storage and release from mast cells: the contributions of inorganic cations, pH, heparin and histamine

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Abstract

Chymase, the major chymotryptic proteinase of human mast cells, can be released in substantial quantities following mast cell activation. As this enzyme is stored in the secretory granules in its fully active form, we have investigated various factors which might regulate its activity in storage and upon release. Chymase was purified from human skin by high salt extraction, cetylpyridinium chloride precipitation, heparin agarose affinity chromatography and gel filtration. Neither the addition of Mg^{2+} or Ca^{2+} (0.3–10 mM) nor their sequestration by EDTA had any effect on the rate of cleavage of the synthetic substrate N-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide. Monovalent cations (Na^+, K^+) enhanced enzyme activity, but only at non-physiological concentrations (0.5–3.0 M), suggesting an ionic strength effect. At constant $I = 0.15$, enzyme activity was strongly pH-dependent: at pH 5.5 (the approximate pH of the mast cell granule) the activity was only 10% of that at pH 7.5 (the approximate pH of the extracellular space). Heparin, which is stored with chymase in the mast cell granule, accentuated this difference by enhancing activity at pH 7.5 by 33% and depressing it at pH 5.5 by 40%. Histamine at concentrations up to 50 mM ($I = 0.15$) had little effect on chymase activity at either pH, although high concentrations did attenuate the actions of heparin. It is concluded that pH and the interaction with heparin are central to the regulation of chymase activity within the granule and following release.

Keywords: Chymase; Mast cell proteinase; Mast cell granule; Heparin; Enzyme regulation

1. Introduction

Chymase (EC 3.4.21.39) is a chymotrypsin-like serine proteinase stored and secreted by a major subpopulation of mast cells [1,2]. Its absence or presence has been used to classify mast cells into two subsets; the MC_T cells which contain only tryptase (a trypsin-like serine proteinase), and the MC_{TC} cells, which contain both tryptase and chymase [3]. These two sets of cells differ both in granule content and in tissue distribution; the MC_T cells tend to be most numerous in the mucosal tissues of the respiratory and intestinal tracts, while the MC_{TC} cells predominate in the submucosa and connective tissue and are especially abundant in skin. The amount of chymase in MC_{TC} cells has been estimated at 4.5 pg $cell^{-1}$ [4]. This compares with 1 pg $cell^{-1}$ for cathepsin G and elastase in neutrophils and 10–35 pg $cell^{-1}$ for tryptase in mast cells [2]. Chymase is stored in the same mast cell granules as tryptase, heparin and histamine, and is released upon mast cell activation. Actions which have been attributed to chymase include the highly efficient conversion of angiotensin I to angiotensin II [5], degradation of neurotensin [6], activation of interleukin-1β [7], degradation of interleukin-4 [8], and induction of microvascular leakage and neutrophil accumulation [9,10].

Comparison of the N-terminal sequence of the mature enzyme with the sequence deduced from cDNA indicates that chymase is synthesized as a preproenzyme [11]. The signal peptide is removed to produce the proenzyme which differs from the mature enzyme by the dipeptide Gly-Glu- at the N-terminus, similar to many granulocyte proteinases [12,13]. Studies with various recombinant constructs demonstrated that the proenzyme is catalytically inactive

Abbreviations: Aces, *N*-[2-acetamido]-2-aminoethanesulfonic acid; Amp, 2-amino-2-methylpropan-1-ol; BAPNA, *N*-benzoyl-DL-arginine-*p*-nitroanilide; Mes, 2-[*N*-morpholino]ethanesulfonic acid; SucAAPF-pNA, *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide.

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[14]. However, cleavage of the N-terminal dipeptide with an unfractionated lysate of a lymphoma cell line yielded a fully active enzyme with properties very similar to native chymase. Although the mast cell enzyme responsible for this maturation step has not been identified or characterized, the dipeptidase in this lymphoma cell lysate was optimally active between pH 5 and 6, which prompted the authors to speculate that chymase is packaged to the developing mast cell granule as the inactive proenzyme and subsequently activated during the acidification which accompanies maturation of the granule. A similar scheme has been proposed for cathepsin G and neutrophil elastase, which both require acidification of a post-Golgi compartment for removal of a N-terminal dipeptide prosequence [15,16]. Chymase is then stored in the mast cell granule in an apparently active form, as demonstrated histochemically with chromogenic substrates [17].

It would be highly unusual for a degradative enzyme to be fully active under conditions of storage, especially if it can degrade itself as has been reported for chymase [18]. It is therefore likely that some means other than covalent modification controls the activity of chymase during storage and release. General factors which modulate the activity of enzymes include inorganic cations, ionic strength, and pH. The concentrations of inorganic cations in mast cell granules are not well documented, but a pH of 5.55 ± 0.06 has been reported for granules isolated from rat peritoneal mast cells [19]. Heparin and histamine, which are stored with chymase in the mast cell granule, have been reported to affect the activity of a variety of enzymes. Heparin can activate tissue-type plasminogen activator [20], stabilise mast cell tryptase [21], inhibit phospholipase A_2 [22], alter the substrate specificity of mouse T-cell serine proteinase 1 [23], and alter the susceptibility to physiological inhibitors of thrombin [24] and elastase [25]. Histamine, which is present at an estimated 100 mM within the granule, inhibits tryptase by 83% at only one tenth of that concentration [26]. In the present study, we have systematically examined the potential importance of these various factors on the regulation of chymase activity during storage and following release from mast cells.

2. Materials and methods

2.1. Materials

Heparin agarose was obtained from Muratech (Aylesbury, Bucks., UK) and Sephacryl S-200 from Pharmacia (Milton Keynes, UK). Human neutrophil cathepsin G and heparin (porcine intestinal mucosa, molecular mass range of 13–15 kDa) were purchased from Calbiochem (Nottingham, UK), biotinylated antibodies to mouse IgG, peroxidase-conjugated antibodies to rabbit IgG and streptavidin-horseradish peroxidase conjugate from Dako (High Wycombe, Bucks., UK), and all other reagents from Bio-Rad (Hemel Hempstead, UK), Sigma (Poole, UK) or BDH (Poole, UK). AA5 (anti-tryptase monoclonal antibody) was produced as described previously [27], purified polyclonal IgG raised against cathepsin G in sheep was kindly donated by Dr. D. Burnet (Birmingham, UK), and polyclonal rabbit serum raised against chymase was kindly donated by Dr. N. Schechter (Philadelphia, USA).

2.2. Chymase purification

Skin obtained from amputated limbs and apronectomy operations was passed several times through a meat grinder and extracted three times with low salt buffer (1 mm EDTA, 50 mm Mes, adjusted with NaOH to pH 6.8), then three times with high salt buffer (2.0 M NaCl, 1 mm EDTA, 50 mM Mes (pH 6.8)) [28]. Each high salt extract was mixed with an equal volume of 10% cetylpyridinium chloride and left to stir overnight at 4° C to precipitate proteoglycans [29]. After centrifugation at $30\,000 \times g$ for 30 min, the supernatants were successively filtered through Whatman GF/F and 0.45 μm pore Millipore membranes. The ionic strength was adjusted to 2 M NaCl prior to concentration in a Pellicon tangential flow ultrafiltration system (Millipore, Watford, UK) fitted with a PLGC membrane (10 kDa nominal cut-off). The retentate was diluted 5-fold with 10 mM Mes (pH 6.8), and concentrated again. The system was then washed by recirculating 200 ml Buffer A (0.4 M NaCl, 10 mM Mes (pH 6.8)) for 10 min, and the combined retentate and wash were applied to a 4.5×1.3 cm column of heparin-agarose equilibrated with Buffer A at a flow rate of 0.50 ml min^{-1} cm^{-2}. The column was washed with more than twelve bed volumes of Buffer A followed by an 80 ml gradient from 0.4 to 2.0 M NaCl in 10 mM Mes (pH 6.8). Fractions were pooled and concentrated in C-10 Centricon centrifugal concentrators (Amicon, Stonehouse, Glos., UK) prior to being loaded on an 88×2.5 cm column of Sephacryl S-200 equilibrated with 2.0 M NaCl, 100 mM Mes (pH 6.8). The chymase-containing fractions were pooled and concentrated as above and dispensed into 200 μl samples before storage at −70° C.

2.3. Enzyme assays

Chymase activity during purification was monitored by the hydrolysis of 0.7 mM *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (SucAAPF-pNA) in 1.5 M NaCl, 0.3 M Tris-HCl (pH 8.0), 4.5% dimethylsulfoxide. Most characterisation studies were done in 0.12 M NaCl, 0.05 M Tris-HCl (pH 7.6), 4.5% dimethylsulfoxide. Buffers used for pH profile studies were formulated to maintain a constant ionic strength ($I = 0.15$) [30] and contained either 50 mM acetic acid, 50 mM *N*-[2-acetamido]-2-aminoethanesulfonic acid (Aces), 100 mM Tris, 50 mM NaCl or 100 mM Aces, 52 mM Tris, 52 mM 2-amino-2-methyl-1-propanol (Amp), 50 mM NaCl, adjusted to the appropriate pH

with either HCl or NaOH. Tryptase was assayed using *N*-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) [27], and elastase was assayed with *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide using the same buffer as BAPNA. Assays were performed in microtitre plates which had been previously blocked with 3% gelatine in phosphate-buffered saline containing 0.2% Tween-20 [31]. In all assays, 10 μl of sample was mixed in a total reaction volume of 100 μl. Multiple readings at 410 nm were taken using the kinetics programme of a Dynatech MR5000 platereader (Billingshurst, West Sussex, UK), and reaction rates calculated by linear regression, assuming an extinction coefficient of 8800 M^{-1} cm^{-1} for *p*-nitroaniline and an average diameter of 6.6 mm for each well in the microtitre plate. Kinetic constants were calculated by the Cornish-Bowden direct linear plot method adapted to a spreadsheet program on a PC.

2.4. Other assays

Protein was determined with Brilliant blue G [32] with bovine serum albumin (BSA) as standard. Uronic acids were determined by the carbazole method [33] with glucuronolactone and heparin as standards.

2.5. Electrophoresis and Western blotting

SDS-PAGE was performed on 10% gels. The gels were stained with the Bio-Rad silver stain according to manufacturer's specifications. Western blotting was carried out in a wet transfer system and after blocking with 3% gelatine, blots were probed with the appropriate antibody, and developed by treatment with the corresponding secondary antibody followed, if appropriate, by a streptavidin-horseradish peroxidase conjugate. Colour was developed with diaminobenzidine.

3. Results

3.1. Purification

Chymase binds to proteoglycans at low concentrations of salt, a property that is exploited both in extraction (removal of 80% of extractable proteins with low salt buffer before release of chymase with high salt buffer) and in purification (heparin-agarose affinity chromatography). However, proteoglycans are also extracted by high salt buffer and can interfere with the subsequent affinity chromatography. Cetylpyridinium chloride is commonly used to precipitate proteoglycans and glycosaminoglycans, and has been used successfully in the purification of tryptase from human lung [29]. Its use in the purification of chymase from human skin not only resulted in the precipitation of the proteoglycans, but also of 80% of the total protein, with 85–95% of the chymase remaining in the supernatant.

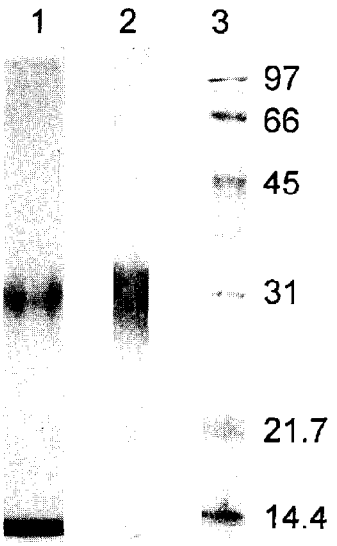

Fig. 1. SDS-PAGE and Western blotting of purified chymase. Lane 1: purified human skin chymase on silver-stained gel (10% T). Lane 2: purified human skin chymase on Western blot probed with rabbit antiserum to human chymase. Lane 3: biotinylated molecular mass standards on Western blot probed with streptavidin-horseradish peroxidase. Molecular mass expressed in kDa.

Chromatography on heparin agarose resolved a major peak of chymotryptic activity, which eluted at 1.1 M NaCl, and two smaller peaks. The major peak, containing 65% of the recovered activity, was further purified by chromatography on Sephacryl S-200, eluting at a K_{av} equivalent to 34 kDa. The final preparation of chymase had a specific activity of 33 μmol min^{-1} mg^{-1} and was devoid of any tryptase or elastase activity. SDS-PAGE followed by silver staining revealed a diffuse band at 30 kDa which reacted with antibodies to chymase in western blots (Fig. 1). Some low molecular mass material, which migrated at the front and reacted weakly with antibodies to chymase, was also present. There was no reaction in western blots with antibodies to tryptase or cathepsin G, though in positive control experiments, these antibodies bound to purified tryptase and cathepsin G, respectively.

3.2. Adsorption to vessel surfaces

Chymase appeared to adsorb strongly to the walls of the microtitre plates used for enzyme assay with a concomitant loss of activity. This tendency to stick to container surfaces has been previously reported [34], and overcome by the inclusion of Triton X-100 in all buffers [14,18,34]. However, as Triton X-100 might affect the properties of chymase under investigation in this study, alternative measures to counteract adsorption were sought. Prior coating of the microtitre plates with blocking agents (Tween-20, BSA, gelatine, skimmed milk powder) [31] was found to enhance enzyme activity, the degree of enhancement (40%) being independent of the blocking agent used. As gelatine gave the lowest background and least interference in other

assays, it was the blocking agent of choice for all subsequent assays.

Chymase activity was also enhanced by the inclusion of BSA or gelatine in the assay medium. The degree of enhancement depended on the purity of the chymase: the more pure the sample, the greater the effect, as if endogenous proteins had a blocking effect comparable to the added protein. For example, in crude extracts, addition of exogenous protein had no effect, while with partially purified enzyme, it stimulated activity in uncoated plates but not in coated plates, and with the final preparations of purified chymase, the addition of BSA or gelatine enhanced activity even in coated plates. This degree of enhancement (20–80%) with highly purified chymase suggests that in the absence of other proteins in solution, prior blocking of the plates is not sufficient to prevent adsorption. Therefore, characterisation studies of chymase routinely included a treatment with BSA (100 μg ml^{-1}) as an additional control.

Loss of chymase activity on storage was also affected by protein concentration. There was relatively little loss of activity when crude extracts were stored frozen. However, the dilute yet highly purified fractions eluted from the Sephacryl S-200 column lost activity very rapidly in storage at −70° C; in one instance, more than 90% of the activity was lost after only 16 days. Concentrating these fractions 15-fold prior to freezing reduced this loss to less than 20% after a month in storage (data not shown).

3.3. Effects of inorganic cations

Under approximately physiological conditions (0.12 M NaCl, 0.05 M Tris-HCl (pH 7.6)), Mg^{2+} and Ca^{2+} had no effect on the activity of purified chymase over the range of 0.3 to 10.0 mM. Similarly, addition of EDTA (10 mM) had no effect on activity, suggesting that even trace levels of divalent cations were not necessary for full activity. The activity obtained under these conditions was substantially less than that observed with the high salt buffer used in routine assays during purification. A dose-response curve showed that activity increased with increasing NaCl concentration to 2 M, and thereafter appeared to reach a plateau to 3 M (Fig. 2). KCl had a similar effect (results not shown). Approximately the same degree of stimulation by NaCl was seen regardless of whether the plates were uncoated, coated with gelatine, or whether Triton X-100 or BSA was present to reduce adsorption to plate walls, strongly suggesting this stimulation is a direct effect of ionic strength on the enzyme, independent of the effects of adsorption to vessel walls.

3.4. Effects of heparin and pH

At mass ratios relative to chymase of 0.1:1 to 30:1 (0.0075 to 2.25 μg ml^{-1}), heparin, but not chondroitin sulfate C or dextran sulfate, enhanced the activity of purified chymase in a dose-dependent manner in the presence of 0.12 M NaCl, 0.05 M Tris-HCl (pH 7.6), although the degree of enhancement (approximately 40% at 30:1 mass ratio) was comparable to that produced by the same amount of BSA or gelatin. Marked differences were, however, observed between heparin and BSA in their effect on the pH profile of the enzyme. At constant ionic strength ($I = 0.15$), all three treatments (no addition, 100 μg ml^{-1} heparin, 100 μg ml^{-1} BSA) produced a monotonic increase in activity with increasing pH from 4.0 to 10.0 with a slight drop at pH 10.5 (Fig. 3). Whereas BSA enhanced chymase activity at all pH values, heparin stimulated activity only at pH values greater than 6.0; below pH 6.0 it depressed it ($P = 0.0001$, $P < 0.0001$, and $P = 0.65$ for pH 5.0, 5.5, and 6.0, respectively, by Student's t-test). Consequently, heparin accentuated the inhibitory effects of low pH. Either in the presence of BSA or in the absence of any additive, the activity of chymase at pH 5.5 (the approximate pH of the mast cell granule) was 10% of the activity at pH 7.5. In the presence of heparin, activity at pH 5.5 was only 5% of the activity at pH 7.5. Incubation for 1 h at 20° C at pH 4.0–6.0 followed by assay at pH 8.0 demonstrated that chymase was stable over this range, and even retained more activity than after incubation at pH 7.5.

The action of these macromolecules was examined in more detail at pH 5.5, 6.0, and 7.5 (Table 1). For all three treatments, the V_{max} decreased with decreasing pH, but whereas the V_{max} of chymase + BSA remained greater than the V_{max} of chymase alone, the V_{max} of chymase + heparin was greater than the V_{max} of chymase alone at pH 7.5, equal to it at pH 6.0, and less than it at pH 5.5. The K_m of the enzyme was also affected by these treatments. Chymase alone had little change in K_m with the decrease in pH, but a marked increase was seen in the presence of both heparin and BSA.

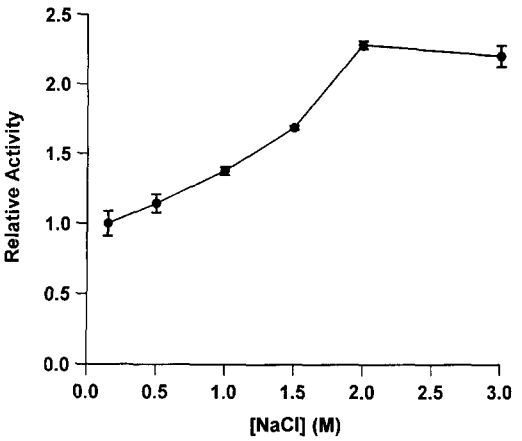

Fig. 2. Effect of NaCl concentration on chymase activity. Purified chymase (75 ng ml^{-1}) was assayed in 0.05 M Tris-HCl (pH 8.0), with SucAAPF-pNA (0.7 mM) as substrate. Values relative to the activity at 0.15 M NaCl are expressed as mean ± S.E. ($n = 3$).

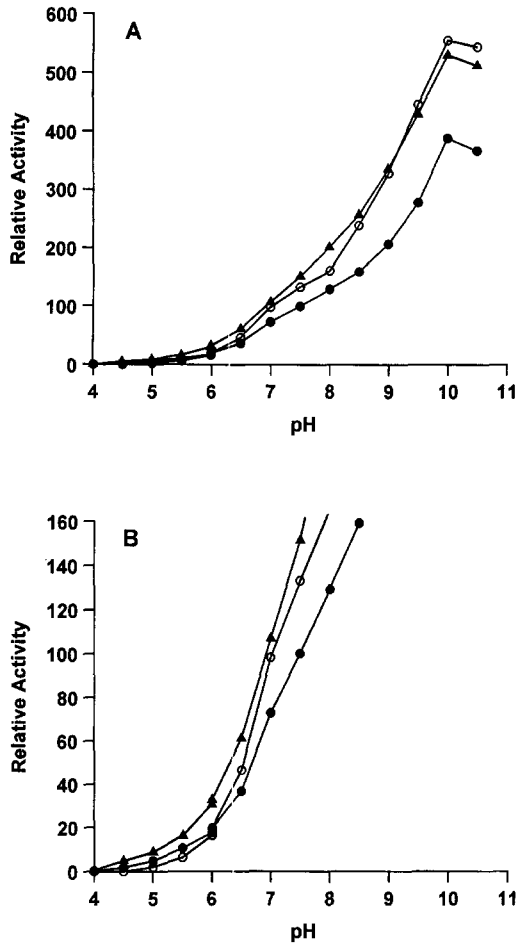

Fig. 3. pH profile of purified chymase: effect of heparin and BSA. (A) Purified chymase (150 ng ml^{-1}) was assayed using 0.7 mM SucAAPF-pNA in acetic acid/Aces/Tris buffer (pH 4.0–6.5) or Aces/Tris/Amp buffer (pH 6.0–10.5)($I = 0.15$) with (●) no addition, (○) 100 μg/ml heparin, or (▲) 100 μg/ml BSA. Activity is expressed as percentage of activity at pH 7.5 with no addition. (B) As (A) but scale expanded to show depression of activity by heparin at low pH. Error bars omitted as S.E.M. values are less than or equal to symbol diameter ($n = 3$).

Table 1
Effect of pH, heparin and BSA on the kinetic constants of purified chymase for a peptide substrate (SucAAPF-pNA)

pH	Addition	K_m (mM)	V_{max} (μmol min^{-1} mg^{-1})
7.5	none	0.80	44
7.5	100 μg/ml heparin	0.98	62
7.5	100 μg/ml BSA	1.04	92
6.0	none	0.85	32
6.0	100 μg/ml heparin	1.23	32
6.0	100 μg/ml BSA	1.23	39
5.5	none	0.90	19
5.5	100 μg/ml heparin	1.60	17
5.5	100 μg/ml BSA	1.87	27

Purified chymase (70 ng ml^{-1} for pH 7.5, 150 ng ml^{-1} for pH 6.0 and 5.5) was assayed without any addition or in the presence of heparin or BSA.Buffers were 50 mM acetic acid, 50 mM Aces, 100 mM Tris, 50 mM NaCl, adjusted to the given pH with either HCl or NaOH ($I = 0.15$). Substrate concentrations ranged from 0.25 to 4.0 mM, $n = 3$ for each combination of [S], pH and addition.

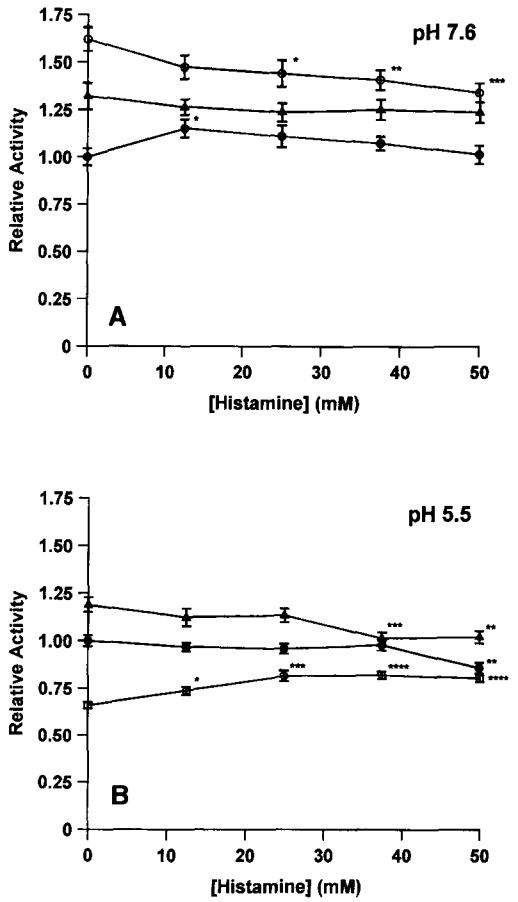

Fig. 4. Effect of histamine concentration on chymase activity in the presence or absence of heparin and BSA. Purified chymase was assayed using 0.7 mM SucAAPF-pNA with (●) no addition, (○) 100 μg ml^{-1} heparin, or (▲) 100 μg ml^{-1} BSA in (A) 0.05 M Tris-HCl (pH 7.6) (enzyme concentration = 0.15 μg ml^{-1}), or (B) 0.05 M acetic acid, 0.05 M Aces, 0.10 M Tris (pH 5.5) (enzyme concentration = 1.2 μg ml^{-1}). The concentration of NaCl and buffer were adjusted to maintain a constant ionic strength ($I = 0.15$) using the formula $I = \frac{1}{2}\Sigma c_i z_i^2$ and the assumption, based on titrametric data, that at pH 5.5 histamine was 13% monovalent and 87% divalent, and at pH 7.6 it was virtually all monovalent. Values relative to the activity with no histamine or other addition are expressed as mean ± S.E. ($n = 3$). Significant difference by Student's t-test from activity in the absence of histamine, but in the presence of the indicated addition: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, **** $P < 0.001$.

3.5. Histamine

At constant ionic strength ($I = 0.15$), histamine had negligible effect on the activity of purified chymase on its own or in the presence of BSA at pH 7.6 (Fig. 4A), and a small inhibitory effect at pH 5.5 (Fig. 4B). The action of histamine in the presence of heparin was more complex: it inhibited the enzyme at pH 7.6 and stimulated it at pH 5.5, but not to the extent that it overrode the stimulating effect of heparin at pH 7.6, or its inhibiting effect at pH 5.5. Histidine (50 mM) as control had no significant effect on activity in any of the treatments.

4. Discussion

The pH profile of chymase is similar to that which has been reported for chymotrypsin with a steadily rising, almost sigmoidal increase with increasing pH [35]. Such a profile is indicative of activity being dependent on the ionization state of a single amino acid residue, presumably at the active site. In both the presence and absence of heparin, chymase was quite stable at acidic pH, which is what would be expected for an enzyme stored under such conditions in the mast cell granule. That it proved to be more stable at pH 5.5 than at pH 7.5 could be due to reduced autodegradation resulting from lowered activity. Autodegradation of chymase has been reported [18] and could account for the low molecular mass bands seen on SDS-PAGE gels (Fig. 1).

Heparin was found to enhance the pH-dependent regulation of chymase by increasing activity at pH 7.5 by 33% and decreasing it at pH 5.5 by 40%. This was achieved by both a decrease in V_{max} relative to enzyme alone and an increase in K_m as pH decreased, while maintaining hyperbolic kinetics. Thus, at pH 5.5 heparin behaved as a mixed inhibitor, even though at pH 7.5 it behaved as an activator. In contrast to its potent inhibition of tryptase [26], histamine has little effect of its own on chymase activity, but does appear to modulate the action of heparin on chymase, reducing both its ability to stimulate enzyme activity at pH 7.6 and its ability to inhibit it at pH 5.5. These observations could be due to a weakening of the interaction between heparin and chymase. Such an effect would have to be specific to histamine as histidine had no effect and all experiments were conducted at constant ionic strength.

BSA, in contrast to heparin, enhanced activity at all pH values. It consistently had a higher V_{max} than enzyme alone, which is what would be expected if it were increasing the total amount of chymase in solution by blocking adsorption to the microtitre plate (i.e., as $V_{max} = k_{cat}[E_{TOT}]$, it acts by increasing $[E_{TOT}]$ rather than k_{cat}). However, as a protein, BSA is a potential substrate for chymase and rat serum albumin has been reported to be cleaved by rat chymase [36]. Therefore, it would be expected to behave as a competitive inhibitor when monitoring the hydrolysis of a chromogenic substrate and raise the K_m for that substrate. This was, indeed, observed, but it is not clear why the K_m values should be nearly the same as those derived in the presence of heparin.

Modest stimulation by heparin for the cleavage of synthetic peptide substrates has been noted for dog [37], rat [38] and human [39] chymase. Evaluation of the effect of heparin is rendered difficult by the tendency of chymase to adsorb to surfaces. Adsorption to polystyrene surfaces should be primarily hydrophobic in character and any blocking agents would be expected to be amphipathic. Although proteins and detergents meet this criterion, sulfated polysaccharides do not. Accordingly, chondroitin sulfate C and dextran sulfate did not enhance the activity of chymase, whereas heparin did. Furthermore, histamine had no significant effect on the action of BSA on chymase relative to its action on the enzyme alone. Taken together, these results suggest that the mechanism of action of heparin on chymase is different from that of BSA, and that the stimulation with peptide substrates seen at pH 7.5 could be physiologically relevant.

The modest effect of heparin on chymase activity towards peptides may be a consequence of their small size. With larger substrates, more dramatic results have been reported. Heparin was found to inhibit the degradation of proteoglycans by dog chymase and also the secretagogue activity of this enzyme on cultured bovine airway gland serous cells [37]. With rat chymase, also known as rat mast cell proteinase 1 (RMCP-1), heparin produced a ninefold increase in the enzyme's ability to inactivate thrombin [38]. Heparin has been found to affect the action of human chymase on interstitial procollagenase in two ways: it accelerated the cleavage step which forms active enzyme and increased the specificity of chymase so that there was no further degradation of the collagenase [40].

The results of the present study, together with the results of studies of the expression of recombinant chymase [14], lead us to make the following proposals for the control of enzyme activity during the synthesis, storage, and release of chymase. During synthesis, the nascent polypeptide chain is injected into the lumen of the endoplasmic reticulum with the cleavage of the signal peptide to produce the proenzyme. This catalytically inactive prochymase is processed through the Golgi apparatus and directed to the developing mast cell granule, with the N-terminal propeptide possibly serving as a tag for intracellular trafficking. As the granule matures, acidification occurs, activating an N-terminal dipeptidase to remove the propeptide. The mature chymase, in tight association with heparin, is kept relatively inactive by the acidic pH. The heparin also restricts diffusion of the chymase, further limiting hydrolysis of any other protein present. It has also been demonstrated that isolated mast cell granular matrices expand in the presence of monovalent cations and condense in the presence of divalent cations so that at pH 5.5, histamine, being mostly in the divalent form, causes a condensation of the heparin matrix [41], thus restricting diffusion to an even greater extent.

Upon mast cell degranulation, the matrix expands rapidly, partly due to the neutralisation of histamine by the increase in pH, partly due to displacement of histamine by sodium ions [41], and possibly abetted by local electric potentials [42]. Chymase is rapidly activated by the increase in pH, and although histochemical evidence suggests that it remains bound to the matrix [39], the expansion of the matrix allows for the diffusion of substrates to the fully active enzyme. Locally high concentrations of histamine would have very little effect on activity. Further work is needed to confirm the various steps in this scheme, especially the covalent processing of the prochymase in

the Golgi apparatus and maturing granule. It will also be important to establish the effects of heparin on activity of the enzyme towards physiological substrates and on the inactivation of chymase by serum and tissue proteinase inhibitors.

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