Interaction of mucopolysaccharides with chymotrypsin*

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Chondroitin sulphates A, B and C form complexes with chymotrypsin which enhance the hydrolytic activity of the enzyme towards glutaryl-L-phenylalanine-p-nitroanilide as much as 270%, as in the case of chondroitin sulphate A. Hyaluronic acid, a non-sulphated mucopolysaccharide, at a weight ratio of 6.67:1 to the enzyme, does not influence the rate of cleavage of the substrate by chymotrypsin. Proflavin difference spectra and ultra-violet spectra suggest that a complex is formed between chymotrypsin and hyaluronic acid, as well as chondroitin sulphates A, B and C. The proflavin molecule is not displaced from the active site of the enzyme upon association with the mucopolysaccharides. When the mucopolysaccharide–chymotrypsin complexes are electrophoresed on analytical polyacrylamide disc gels at pH 8.9, the complexes are visualized by toluidine blue (for mucopolysaccharide), coomassie brilliant blue (for protein) and by the zymogram technique (with glutaryl-L-phenylalanine-β-naphthylamide), at a position between that for chymotrypsin alone and that for the mucopolysaccharide alone.

INTRODUCTION

The interaction between heparin and serine proteases such as thrombin[1–3] and plasmin[4] have been studied due to the probable role of these reactions in the blood clotting system. The complex between heparin and trypsin or chymotrypsin has been studied[5,6]. These complexes have been probed for their possible use as fibrinolytic agents[7–10] and anti-inflammatory agents[11].

The interaction between proteolytic enzymes and other mucopolysaccharides has not been extensively studied. Chondroitin sulphate has been shown to inhibit pepsin under acidic conditions[12,13]. This interaction probably protects the lining of the stomach since chondroitin sulphate A and other sulphated glycoproteins are secreted from the fundus[14]. Under certain conditions, however, sulphated mucopolysaccharides have been shown to activate pepsinogen to pepsin[15]. This interaction has been implicated in peptic ulcerogenesis[15].

Sulphated mucopolysaccharides have been detected in intestinal mucus[16] and the intestinal mucosa[16,17], being localized in the mucus secreting goblet cells[18] and mast cells[19]. The interaction between sulphated mucopolysaccharides and pancreatic chymotrypsin and trypsin in the intestinal tract is probably important physiologically since these proteases bind to the intestinal mucosa[20], inactivate membrane-bound brush border enzymes[21], and pass through the intestinal mucosa into the mesenteric vein in an enzymatically and immunologically active form[22].

Hyaluronic acid (HA), chondroitin sulphate A. (CS-A, chondroitin-4-sulphate), chondroitin sulphate B (CS-B, dermatan sulphate), chondroitin sulphate C (CS-C, chondroitin-6-sulphate) and heparin represent a series of mucopolysaccharides which vary in the degree of sulphation and the relative concentrations of sugar residues. Hyaluronic acid, CS-A and CS-C contain glucuronic acid as the only uronic acid while CS-B and heparin contain iduronic acid as the major uronic acid and glucuronic acid as the minor uronic acid. HA contains non-sulphated *N*-acetylglucosamine while CS-A, CS-B and CS-C contain sulphated *N*-acetylgalactosamine residues. This series of mucopolysaccharides offers an opportunity to try to understand the binding between mucopolysaccharides and proteolytic enzymes.

The interaction between heparin and chymotrypsin has previously been studied[6] using proflavin difference spectroscopy, ultra-violet spectroscopy, electrophoresis and enzymatic studies. By the use of these techniques, a complex between heparin and chymotrypsin was detected which was enzymatically more active than chymotrypsin alone towards the synthetic substrates, glutaryl-L-phenylalanine-β-naphthylamide and glutaryl-L-phenylalanine-*p*-nitroanilide. Using these same methods, we report here that CS-A, CS-B, CS-C and hyaluronic acid form complexes with chymotrypsin which are similar to that formed between heparin and chymotrypsin. CS-A, CS-B and CS-C, like heparin[6], induce an enhancement of chymotryptic activity. Hyaluronic acid has no effect on the enzymatic activity of chymotrypsin. A preliminary report has appeared elsewhere[23].

EXPERIMENTAL

CS-A, CS-B, CS-C and HA from whale cartilage, pig skin, shark cartilage and vitreous humour, respectively, were obtained from Sigma Chemical Corporation, St. Louis, Mo.

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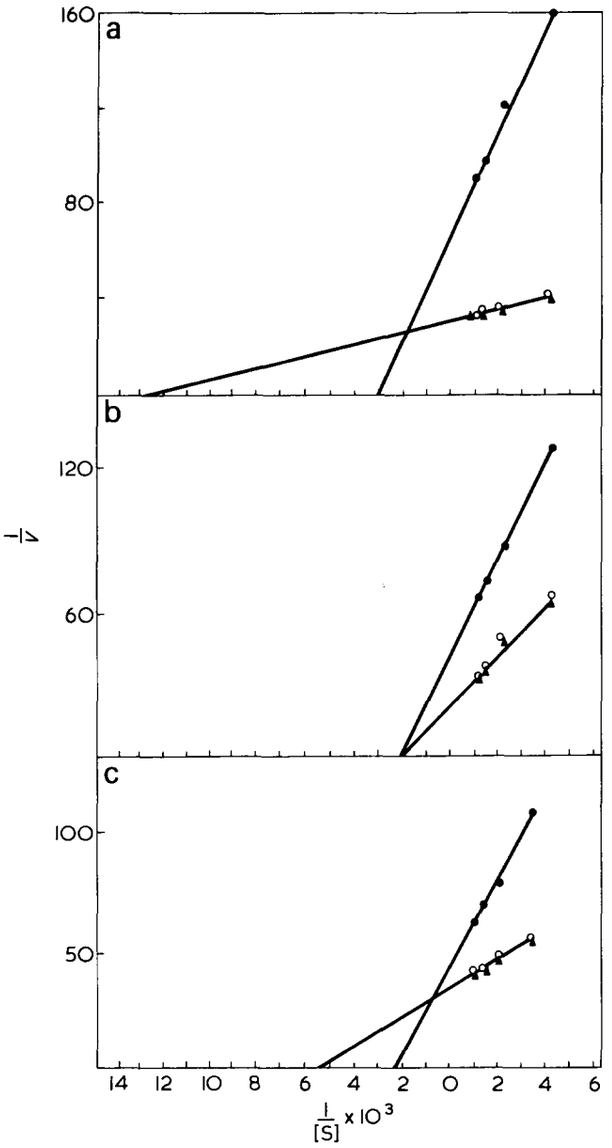

Figure 1 Influence of chondroitin sulphate A, B and C on chymotryptic hydrolysis of *N*-glutaryl-L-phenylalanine-*p*-nitroanilide (GPANA). One ml solutions containing chymotrypsin plus chondroitin sulphate A, B or C in 1 mM HCl were incubated at 25°C for 10 min. To 0.2 ml of these mixtures, 1.0 ml of 1.0×10^{-3} M GPANA in 0.05 M Tris Buffer, pH 7.6, containing 0.02 M $CaCl_2$ at 25°C was added. After 10 min, the reaction was terminated by the addition of 0.2 ml of 30% acetic acid. The amount of *p*-nitroaniline released was determined spectrophotometrically at 410 nm. (a) ●, 50 μg Chymotrypsin; ○, 50 μg chymotrypsin plus 140 μg chondroitin sulphate A; ▲, 50 μg chymotrypsin plus 200 μg chondroitin sulphate A. (b) ●, 50 μg Chymotrypsin; ○, 50 μg chymotrypsin plus 200 μg chondroitin sulphate B; ▲, 50 μg chymotrypsin plus 140 μg chondroitin sulphate B. (c) ●, 50 μg Chymotrypsin; ○, 50 μg chymotrypsin plus 250 μg chondroitin sulphate C; ▲, 50 μg chymotrypsin plus 200 μg chondroitin sulphate C

Electrophoresis grade acrylamide and *N,N* methylene-bis-acrylamide were purchased from Eastman Kodak, Rochester, New York. Proflavin sulphate, glutaryl-L-phenyl-alanine-*p*-nitroanilide (GPANA), glutaryl-L-phenylalanine-β-naphthylamide (GPNA) were obtained from Schwarz/Mann, Orangeburg, New York. Three times crystallized chymotrypsin was purchased from Worthington Biochemical Corporation, Freehold, New Jersey.

The enzymatic activity of chymotrypsin in the presence of the various mucopolysaccharides was determined using GPANA as the substrate according to the procedure of Erlanger *et al.*[24]. This assay was carried out using 0.05 M Tris Buffer, pH 7.6 containing 0.02 M $CaCl_2$ at 25°C.

Ultra-violet spectra of chymotrypsin, the individual mucopolysaccharides, each mucopolysaccharide plus chymotrypsin, as well as, the difference spectra of the various mucopolysaccharides plus chymotrypsin vs. the corresponding mucopolysaccharide, chymotrypsin or both, each in a separate cuvette at the appropriate concentrations, were obtained in 1 mM HCl and in 0.010 M Tris Buffer, pH 7.6 using a Beckman Acta IV Spectrophotometer. Proflavin spectra in the visible range were obtained using 3.0×10^{-5} M proflavin in 0.010 M Tris Buffer, pH 7.6 solutions.

Polyacrylamide disc gel electrophoresis was carried out under basic conditions according to the procedure of Davis[25]. Protein and mucopolysaccharide molecules were detected on the gels using coomasie brilliant blue[26] and toluidine blue[27], respectively. Chymotryptic activity was visualized using a two-step procedure. The gels were first incubated at 37°C for 2 h in a solution prepared by dissolving GPNA first in methyl cellosolve, and then diluting the solution with 0.04 M Tris Buffer, pH 7.6. The ratio of GPNA: Tris Buffer:methyl cellosolve was 3 mg:3 ml:3 ml. In the second step, the hydrolysed naphthylamine was coupled to fast garnet GBC by placing the gels in 0.14% fast garnet GBC solution (in distilled water) for 1 h.

RESULTS

Figures 1 and *2* show the influence of mucopolysaccharides on the chymotryptic hydrolysis of GPANA. CS-A, CS-B and CS-C act as non-essential activators of chymotrypsin while HA at weight ratios as high as 6.67:1 did not affect enzymatic activity. The CS-A–chymotrypsin complex and the CS-C–chymotrypsin complex act very similarly, kinetically, as expected since these molecules differ only in

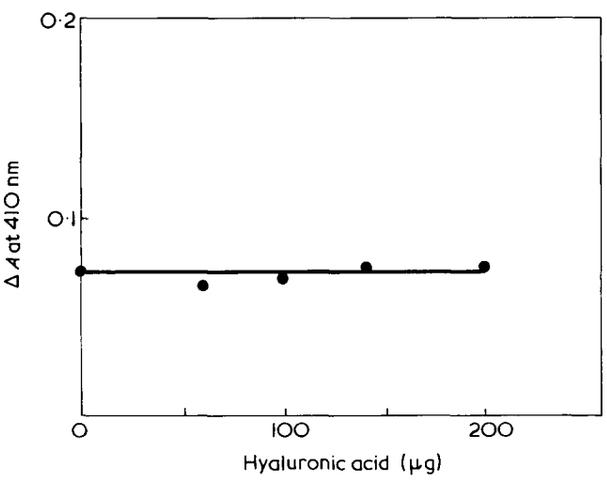

Figure 2 Effect of hyaluronic acid on chymotryptic hydrolysis of *N*-glutaryl-L-phenylalanine-*p*-nitroanilide (GPANA). Aliquots containing 1000, 700, 500 and 300 μg of hyaluronic acid in 1 mM HCl were incubated for 10 min at 25°C with 700 μg of chymtrypsin in 1 mM HCl. To 0.2 ml of these mixtures, 1.0 ml of 1.0×10^{-3} M GPANA in 0.05 M Tris Buffer, pH 7.6, containing 0.02 M $CaCl_2$ at 25°C were added. After 10 min the reaction was terminated by the addition of 0.2 ml of 30% acetic acid. The amount of *p*-nitroaniline released with determined spectrophotometrically at 410 nm

Table 1 Kinetic constants for the hydrolysis of glutaryl-L-phenylalanine-*p*-nitroanilide by chondroitin sulphate A, B and C and heparin plus chymotrypsin complexes. Values for K_A, α, and β were determined from Lineweaver–Burk plots of data gathered using the GPANA assay for chymotryptic activity of Erlanger *et al.*[24]. The assays were carried out at 25°C in 0.05 M Tris chloride buffer, pH 7.6, containing 0.02 M $CaCl_2$. The K_S calculated for chymotrypsin was 4.0×10^{-4} M

Mucopolysaccharide	$K_A \times 10^6$ M	α	β
CS-A	1.06	0.318	1.98
CS-B	0.99	0.992	1.64
CS-C	1.18	0.475	1.21
Heparin[6]	4.79	0.141	1.03

the position of the sulphate group on the *N*-acetylgalactosamine residues. The CS-B–chymotrypsin complex kinetically is quite distinct. The order of enhancement of chymotryptic activity is CS-A > CS-B > CS-C > HA.

Kinetic constants can be calculated for the CS-A, CS-B and CS-C plus chymotrypsin complexes by assuming the mucopolysaccharides function kinetically according to the general scheme for non-essential activation given by the equations[28]:

$$\begin{array}{ccccc} E + S & \overset{K_S}{\rightleftharpoons} & ES & \xrightarrow{k_p} & E + P \\ + & & + & & \\ A & & A & & \\ K_A \Big\| & & \Big\| \alpha K_A & & \\ EA + S & \overset{\alpha K_S}{\rightleftharpoons} & ESA & \xrightarrow{\beta K_p} & EA + P. \end{array}$$

Values for α, β, and K_A are given in *Table 1*. The calculated values for K_A indicate that chymotrypsin has a greater affinity for the mucopolysaccharides than for the substrate GPANA. The enhancement of activity is mainly due to an increase in the rate of reaction of the ESA complex over the ES complex which shifts the equilibrium towards the ESA complex. CS-A and CS-C shift the equilibrium mainly through the ES + A ⇌ ESA pathway while CS-B shifts nearly equal amounts of the enzyme through the EA + S ⇌ ESA and the ES + A ⇌ ESA pathways.

Ultra-violet spectra of the mucopolysaccharides plus chymotrypsin in 1 mM HCl and 0.010 M Tris Buffer are given in *Figures 3* and *4*, respectively. In the presence of 1 mM HCl, all mucopolysaccharides, with the exception of CS-C, altered the absorption of the aromatic residues of chymotrypsin in the 270–290 nm region. However, no change in the 270–290 mm region was observed with 0.01 M Tris Buffer, pH 7.6. There was a greater effect on the spectra in the 220 nm region at the lower pH due to the complex between the mucopolysaccharides and chymotrypsin. These spectra changes, seen as a result of complex formation, were not due mainly to sulphate interactions with the positively charged amino-acid residues of chymotrypsin since the spectra of hyaluronic acid, which is not sulphated, plus chymotrypsin was nearly the same as the spectra for the sulphated mucopolysaccharide–chymotrypsin complexes.

Figure 5 shows the proflavin difference spectra of chymotrypsin plus the various mucopolysaccharides. The proflavin molecule was not removed from the active site of the chymotrypsin molecule upon complex formation with the chondroitin sulphate molecules or the hyaluronic acid molecule, since the absorbance at 465 nm, a wavelength which is characteristic of the proflavin chymotrypsin bond[29], was not diminished. It was enhanced. This indicated that the mucopolysaccharides were bound to the chymotrypsin molecule outside the active site.

Similar electrophoretic patterns were seen when HA, CS-A, CS-B, and CS-C were electrophoresed with chymo-

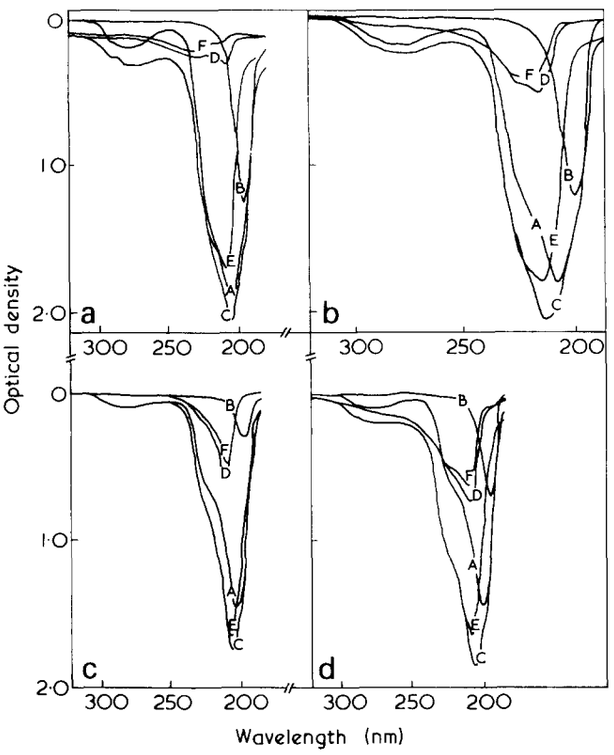

Figure 3 Ultra-violet spectra of chondroitin sulphate A, B, C and hyaluronic acid plus chymotrypsin in 1 mM HCl. Ultra-violet spectra of the mucopolysaccharides, the mucopolysaccharides plus chymotrypsin and chymotrypsin were determined in 1 mM HCl using a Beckman Acta M IV Spectrophotometer. (a) A, Chymotrypsin (100 μg/ml); B, chondroitin sulphate A (300 μg/ml); C, chymotrypsin (100 μg/ml) plus chondroitin sulphate A (300 μg/ml), D, chymotrypsin (1000 μg/ml) plus chondroitin sulphate A (300 μg/ml) vs. chymotrypsin (100 μg/ml); E, chymotrypsin (100 μg/ml) plus chondroitin sulphate A (300 μg/ml) vs. chondroitin sulphate A (300 μg/ml); F, chymotrypsin (100 μg/ml) plus chondroitin sulphate A (300 μg/ml) vs. chymotrypsin (100 μg/ml) and chondroitin sulphate A (300 μg/ml). (b) A, chymotrypsin (100 μg/ml); B, chondroitin sulphate B (300 μg/ml); C, chymotrypsin (100 μg/ml) plus chondroitin sulphate B (300 μg/ml); D, chymotrypsin (100 μg/ml) plus chondroitin sulphate B (300 μg/ml) vs. chymotrypsin (100 μg/ml); E, chymotrypsin (100 μg/ml) plus chondroitin sulphate B (300 μg/ml) vs. chondroitin sulphate B (300 μg/ml); F, chymotrypsin (100 μg/ml) plus chondroitin sulphate B (300 μg/ml) vs. chymotrypsin (100 μg/ml) and chondroitin sulphate B (300 μg/ml). (c) A, Chymotrypsin (50 μg/ml); B, chondroitin sulphate C (300 μg/ml); C, chymotrypsin (50 μg/ml) plus chondroitin sulphate C (300 μg/ml); D, chymotrypsin (50 μg/ml) plus chondroitin sulphate C (300 μg/ml) vs. chymotrypsin (50 μg/ml); E, chymotrypsin (50 μg/ml) plus chondroitin sulphate C (300 μg/ml) vs. chondroitin sulphate C (300 μg/ml); F, chymotrypsin (50 μg/ml) plus chondroitin sulphate C (300 μg/ml) vs. chymotrypsin (50 μg/ml) and chondroitin sulphate C (300 μg/ml). (d) A, Chymotrypsin (50 μg/ml); B, hyaluronic acid (200 μg/ml); C, chymotrypsin (50 μg/ml) plus hyaluronic acid (200 μg/ml); D, chymotrypsin (50 μg/ml) plus hyaluronic acid (200 μg/ml) vs. chymotryspin (50 μg/ml); E, chymotrypsin (50 μg/ml) plus hyaluronic acid (200 μg/ml) vs. hyaluronic acid (200 μg/ml); F, chymotrypsin (50 μg/ml) plus hyaluronic acid (200 μg/ml) vs. chymotrypsin (50 μg/ml) and hyaluronic acid (200 μg/ml)

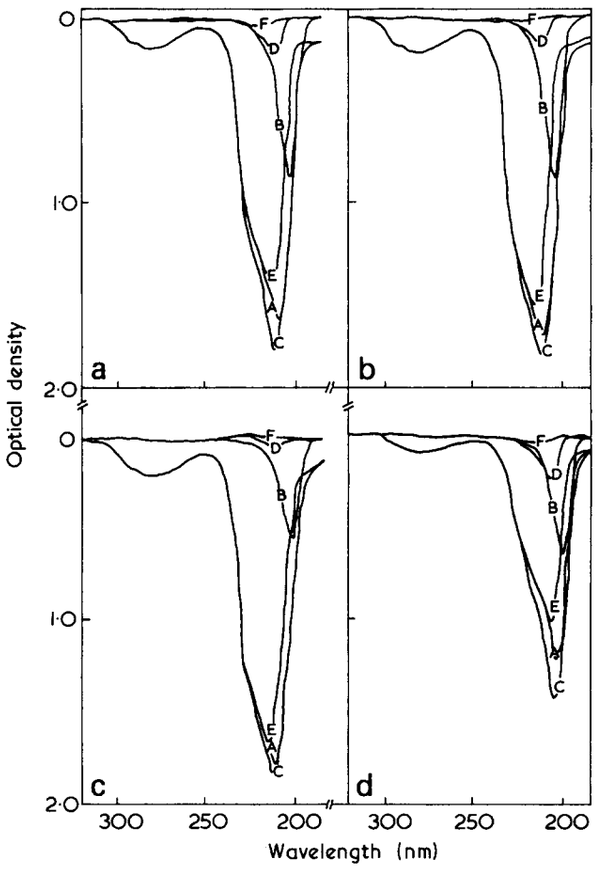

Figure 4 Ultra-violet spectra of chondroitin sulphate A, B, C and hyaluronic acid plus chymotrypsin in 0.010 M Tris Buffer, pH 7.6. Ultra-violet spectra of the mucopolysaccharides, the mucopolysaccharides plus chymotrypsin and chymotrypsin were determined in 0.01 M Tris Buffer, pH 7.6. The mucopolysaccharides and chymotrypsin were dissolved in 0.1 mM HCl and then diluted to 3 ml with 0.01 M Tris Buffer, pH 7.6. (a) A, Chymotrypsin (100 μg/ml); B, chondroitin sulphate A. (300 μg/ml); C, chondroitin sulphate A (300 μg/ml) plus chymotrypsin (100 μg/ml); D, chondroitin sulphate A (300 μg/ml) plus chymotrypsin (100 μg/ml) vs. chymotrypsin (100 μg/ml); E, chondroitin sulphate A (300 μg/ml) plus chymotrypsin (100 μg/ml) vs. chondroitin sulphate A (300 μg/ml); F, chondroitin sulphate A (300 μg/ml) plus chymotrypsin (100 μg/ml) vs. chondroitin sulphate A (300 μg/ml) and chymotrypsin (100 μg/ml). (b) A, Chymotrypsin (100 μg/ml); B, chondroitin sulphate B (300 μg/ml); C, chondroitin sulphate B (300 μg/ml) plus chymotrypsin (100 μg/ml); D, chondroitin sulphate B (300 μg/ml) plus chymotrypsin (100 μg/ml) vs, chymotrypsin (100 μg/ml); E, chondroitin sulphate B (300 μg/ml) plus chymotrypsin (100 μg/ml) vs. chondroitin sulphate B (300 μg/ml); F, chondroitin sulphate B (300 μg/ml) plus chymotrypsin (100 μg/ml) vs. chondroitin sulphate B (300 μg/ml) and chymotrypsin (100 μg/ml). (c) A, Chymotrypsin (100 μg/ml); B, chondroitin sulphate C (300 μg/ml); C, chondroitin sulphate C (300 μg/ml) plus chymotrypsin (100 μg/ml); D, chondroitin sulphate C (300 μg/ml) plus chymotrypsin (100 μg/ml) vs. chymotrypsin (100 μg/ml); E, chondroitin sulphate C (300 μg/ml) plus chymotrypsin (100 μg/ml) vs. chondroitin sulphate C (300 μg/ml); F, chondroitin sulphate C (300 μg/ml) plus chymotrypsin (100 μg/ml) vs. chondroitin sulphate C (300 μg/ml) and chymotrypsin (100 μg/ml). (d) A, chymotrypsin (50 μg/ml); B, hyaluronic acid (200 μg/ml); C, hyaluronic acid (200 μg/ml) plus chymotrypsin (50 μg/ml); C, hyaluronic acid (200 μg/ml) plus chymotrypsin (50 μg/ml) vs. chymotrypsin (50 μg/ml); E, hyaluronic acid (200 μg/ml) plus chymotrypsin (50 μg/ml) vs. hyaluronic acid (200 μg/ml); F, hyaluronic acid (200 μg/ml) plus chymotrypsin (50 μg/ml) vs. hyaluronic acid (200 μg/ml) and chymotrypsin (50 μg/ml)

trypsin. *Figure 6* shows the electrophoretic pattern obtained for CS-A plus chymotrypsin. Complexes were formed between these sulphated mucopolysaccharides and chymotrypsin which migrated to positions intermediate to that of chymotrypsin and the individual mucopolysaccharides. The hydrolysis of GPANA by the complex was visually greater than that by chymotrypsin alone, thereby qualitatively confirming the stimulation of chymotryptic activity observed with GPANA.

DISCUSSION

CS-A, CS-B, CS-C and HA form complexes with chymotrypsin at pH 7.6 and 3.0. Ultra-violet and proflavin spectral studies and enzymatic activity determinations using GPANA as a substrate, indicate that the conformation of the chymotrypsin molecule is altered progressively as the carboxyl to sulphate ratio on the mucopolysaccharide changes. The position of the sulphate group on the mucopolysaccharide

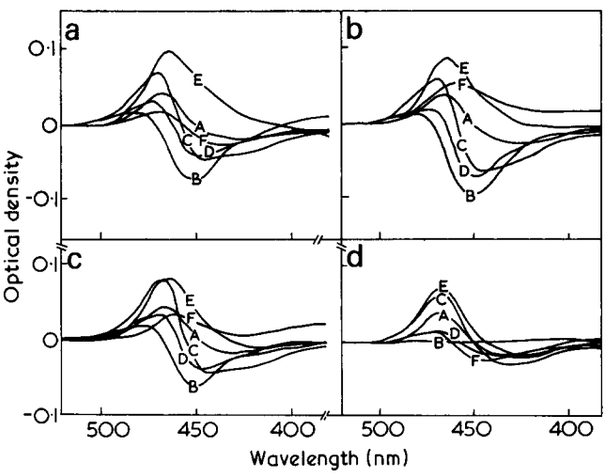

Figure 5 Proflavin difference spectra of chondroitin sulphate A, B, C and hyaluronic acid plus chymotrypsin. Proflavin difference spectra of the individual mucopolysaccharides, the individual mucopolysaccharides plus chymotrypsin and chymotrypsin were determined in 0.01 M Tris Buffer, pH 7.6, containing 3.0×10^{-5} M proflavin. (a) A, Chymotrypsin (133 μg/ml); B, chondroitin sulphate A (400 μg/ml); C, chondroitin sulphate A (400 μg/ml) plus chymotrypsin (133 μg/ml); D, chondroitin sulphate A (400 μg/ml) plus chymotrypsin (133 μg/ml) vs. chymotrypsin (133 μg/ml); E, chondroitin sulphate A (400 μg/ml) plus chymotrypsin (133 μg/ml) vs. chondroitin sulphate A (400 μg/ml); F, chondroitin sulphate A (400 μg/ml) plus chymotrypsin (133 μg/ml) vs, chymotrypsin (133 μg/ml) and chondroitin sulphate A (400 μg/ml). (b) A, Chymotrypsin (133 μg/ml); B, chondroitin sulphate B (400 μg/ml); C, chondroitin sulphate B (400 μg/ml) plus chymotrypsin (133 μg/ml); D, chondroitin sulphate B (400 μg/ml plus chymotrypsin (133 μg/ml) vs. chymotrypsin (133 μg/ml); E, chondroitin sulphate B (400 μg/ml) plus chymotrypsin (133 μg/ml) vs. chondroitin sulphate B (400 μg/ml); F, chondroitin sulphate B (400 μg/ml) plus chymotrypsin (133 μg/ml) vs. chymotrypsin (133 μg/ml) and chondroitin sulphate B (400 μg/ml). (c) A, Chymotrypsin (133 μg/ml); B, chondroitin sulphate C (400 μg/ml); C, chondroitin sulphate C (400 μg/ml) plus chymotrypsin (133 μg/ml); D, chondroitin sulphate C (400 μg/ml) plus chymotrypsin (133 μg/ml) vs. chymotrypsin (133 μg/ml); E, chondroitin sulphate C (400 μg/ml) plus chymotrypsin (133 μg/ml) vs. chondroitin sulphate C (400 μg/ml); F, chondroitin sulphate C (400 μg/ml) plus chymotrypsin (133 μg/ml) vs. chymotrypsin (133 μg/ml) and chondroitin sulphate C (400 μg/ml). (d) A, Chymotrypsin (133 μg/ml); B, hyaluronic acid (267 μg/ml); C, hyaluronic acid (267 μg/ml) plus chymotrypsin (133 μg/ml); D, hyaluronic acid (267 μg/ml) plus chymotrypsin (133 μg/ml) vs. chymotrypsin (267 μg/ml); E, hyaluronic acid (267 μg/ml) plus chymotrypsin (133 μg/ml) vs. hyaluronic acid (267 μg/ml); F, hyaluronic acid (267 μg/ml) plus chymotrypsin (133 μg/ml) vs. hyaluronic acid (267 μg/ml) and chymotrypsin (133 μg/ml)

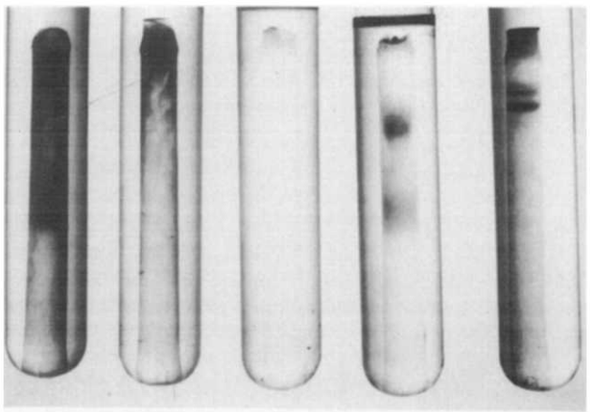

Figure 6 Polyacrylamide disc gel electrophoresis of chondroitin sulphate A, chondroitin sulphate A plus chymotrypsin and chymotrypsin on 7% gels. Polyacrylamide disc gel electrophoresis at pH 8.9 on 7% gels was carried out according to the procedure of Davis[25]. Chondroitin sulphate A, chondroitin sulphate A plus chymotrypsin, and chymotrypsin were placed on the gels, and the gels were electrophoresed at 2 mA per gel for 30 min, followed by 5 mA per gel for approximately 45 min at 0°–5°C. One set of the three gels was immediately placed in 12.5% TCA for 1 h and then in coomassie brilliant blue stain for protein for 30 min. These gels were destained overnight in coomassie brilliant blue destaining solution. Another set of gels was examined for enzymic activity by placing it in glutaryl-L-phenlyalanine-β-naphthylamide for 2 h followed by Fast Garnet GBC for 1 h, and destaining in 7% acetic acid overnight. Left to right: Zymogram: 3 mg chondroitin sulphate A plus 1 mg chymotrypsin; 1 mg chymotrypsin. Coomassie brilliant blue: 120 μg chondroitin sulphate A, 120 μg chondroitin sulphate A plus 40 μg chymotrypsin; 40 μg chymotrypsin

is important because CS-A and CS-C, in which the sulphate groups are found at positions 4 and 6, respectively of the *N*-acetylgalactosamine residues, induce differing degrees of conformational change on the chymotrypsin molecule as observed spectrophotometrically. The sulphate group at position 4 is less available for binding as shown in binding studies using CS-A and CS-C with poly(L-lysine)[30].

The presence of sulphate ions is not a major requirement for the formation of a complex between chymotrypsin and mucopolysaccharides since a complex is formed between chymotrypsin and HA which is spectrophotometrically very similar to the complexes between chymotrypsin and the sulphated mucopolysaccharides. The extent of interaction between chymotrypsin and the mucopolysaccharides does not increase with the number of sulphates nor the availability of the sulphate group. Conversely, it decreases.

In complex formation with poly(L-ornithine), poly(L-lysine) and poly(L-arginine), Gelman and Blackwell[31], in contrast, found that the number of sulphate groups per disaccharide residue on mucopolysaccharides was of major importance. The strength of interaction of these polypeptides at pH 7.0 with the mucopolysaccharides increased according to the order HA < CS-A < heparin sulphate < CS-C < keratan sulphate < CS-B < heparin. These mucopolysaccharides contain approximately 0, 1.0, 1.0, 1.0, 1.0, 1.0, 1.3, 2.3 sulphates per disaccharide, respectively[32]. The extent of interaction between collagen and mucopolysaccharides at pH 3.9–4.4, however, is not simply a function of sulphate ion concentration[33]. The order of increasing interaction between collagen and mucopolysaccharides, using circular dispersion techniques, was determined to be the following: CS-A < keratan sulphate < HA < CS-B < CS-C. The distribution of the charged residues on the collagen and the chymotrypsin molecules in relation to the positions of the sulphate and carboxyl ions and the conformation of the mucopolysaccharides likely play a greater role in determining the extent of interaction than the number of sulphate ions present.

The presence of sulphate groups appear, however, to be important in determining whether the enzymic activity of chymotrypsin is affected by the mucopolysaccharides. The sulphated mucopolysaccharides alter the rate of hydrolysis of GPANA by chymotrypsin, while hyaluronic acid does not alter the hydrolytic activity of chymotrypsin. CS-A, whose sulphate group is least accessible for interaction of the mucopolysaccharides studied, increased the enzymatic activity of chymotrypsin as much as 270% while heparin, which has an average of 2.3 sulphate groups per disaccharide, enhances the activity of chymotrypsin only 70%, at best[6].

The proflavin spectral studies indicate that the mucopolysaccharides bind at a site removed from the active site of chymotrypsin. The mucopolysaccharides might increase the enzyme activity of chymotrypsin in several ways.

(a) By altering the equilibrium between active and inactive molecules of chymotrypsin. At pH 7.0, 85% of the chymotrypsin molecules are in the active conformation and 15% are in the inactive conformation[34]. If the sulphated mucopolysaccharides were simply altering this equilibrium between active and inactive conformations, the maximum increase in activity would be 18% which is considerably below the 270% increase in activity noted for CS-A.

(b) By altering the microenvironment around the chymotrypsin molecule making the active site more attractive to the substrate. The mucopolysaccharides may block some sites at which GPANA would interact with on the outside of the enzyme, leaving only the active site for the substrate molecule to bind. At pH 7.6, GPANA would be negatively charged, such that it would not interact with the mucopolysaccharide.

(c) By altering the conformation of the active site of chymotrypsin. Both the proflavin and the ultra-violet spectra indicate that the mucopolysaccharides alter the conformation of the chymotrypsin. They probably change the conformation of the active site thereby facilitating increased interaction between the substrate and chymotrypsin.

The non-essential activation of chymotrypsin observed upon complex formation with the sulphated mucopolysaccharides most likely is due to a combination of these three factors, with (c), being the major contributing factor.

Chymotrypsin probably interacts with the small intestine epithelial cells as a mucopolysaccharide–chymotrypsin complex. This interaction may be important in control of the production of epithelial cells, since these cells have a high turnover rate[35] and proteolytic enzymes have been shown to be effective mitogens of normal cells in culture[36,37].

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