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INTERACTION OF THE CHYMOTRYPSIN- AND ELASTASE-LIKE ENZYMES OF THE HUMAN GRANULOCYTE WITH GLYCOSAMINOGLYCANS

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Summary

The interaction of the chymotrypsin- (cathepsin G, EC 3.4.21.20) and elastase-like (EC 3.4.21.11) enzymes of the human granulocyte with glycosaminoglycans of biological importance (heparin, heparan sulfate, dermatan sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate and hyaluronic acid) was studied in model experiments. An interaction was established between the glycosaminoglycans and the highly cationic proteases. It manifested itself in the moderate inhibition of the esterolytic activity of the enzymes by glycosaminoglycans at given weight ratios. Glycosaminoglycans were coupled to Sepharose 4B gels by direct activation with CNBr. Binding of proteases on the carbohydrate-coupled gels was investigated. The degree of binding of the elastase-like enzyme was in general: heparin > heparan sulfate > chondroitin-6-sulfate > dermatan sulfate > chondroitin-4-sulfate > hyaluronic acid, whereas that of the chymotrypsin-like enzyme was heparin > chondroitin-6-sulfate > chondroitin-4-sulfate > heparan sulfate > dermatan sulfate > hyaluronic acid. Binding was optimal at pH 6.0. All the glycosaminoglycans, except hyaluronic acid, were capable of binding the elastase- and chymotrypsin-like enzymes. This binding can be of importance with respect to the intracellular latency of enzymes, as well as with respect to the attachment of enzymes to the cell surface and various tissues.

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Abbreviations: Boc-Ala-Np, *N*-*t*-butoxy-carbonyl-L-alanine-*p*-nitrophenyl ester; Bz-Tyr-OEt, *N*-α-benzoyl-tyrosine-ethyl ester; Me_2SO, dimethyl sulfoxide; BPTI, basic pancreatic trypsin inhibitor.

Introduction

Cationic neutral proteases are believed to occur in granulocytes in latent form, i.e., bound to proteoglycans [1]. The glycosaminoglycan content of granulocytes consists mainly of chondroitin-4-sulfate [2,3].

The lysosomal hydrolases are strongly inhibited by glycosaminoglycans [4]. The glycosaminoglycan-lysosomal enzyme interaction appears to be an electrostatic one [5].

The aim of the present model experiments was to clarify the interaction of human granulocyte neutral proteases, chymotrypsin- (cathepsin G, EC 3.4.21.20) and elastase-like (EC 3.4.21.11) enzymes, with glycosaminoglycans of biological importance.

Experimental

Chemicals. *N*-*t*-Butoxy-carbonyl-L-alanine-*p*-nitrophenyl ester was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), *N*-α-L-benzoyl-L-tyrosine-ethyl ester and crystalline egg-white lysozyme from Calbiochem (San Diego, CA, U.S.A.). Sepharose 4B and hyaluronic acid were obtained from Pharmacia (Uppsala, Sweden). Affi-gel 10 was obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Crystalline α-chymotrypsin and trypsin (both from bovine pancreas) and CNBr were from Merck (Darmstadt, F.R.G.), 4-fenil-butylamine (98% pure) from Aldrich-Europe (Beerse, Belgium). Soybean trypsin inhibitor, bovine serum albumin and ovalbumin were purchased from Serva (Heidelberg, F.R.G.). Basic pancreatic trypsin inhibitor was generously gifted by Dr. A. Kelemen (Richter, G. Ltd., Budapest, Hungary). All the other reagents were of analytical grade.

Glycosaminoglycans. All glycosaminoglycan preparations were available at the Department of Medical Physiological Chemistry, Swedish University of Agricultural Sciences (Uppsala, Sweden). Heparin (stage 14) from pig intestinal mucosa was obtained from Inolex Pharmaceutical Division (Park Forest South, IL, U.S.A.) and purified by repeated precipitation with cetylpyridinium chloride from 1.2 M NaCl [6]. Heparan sulfate was preparated from human aorta [7]. Dermatan sulfate, prepared by alkaline copper precipitation from heparin by-products, was generously given by Dr. L. Rodén (University of Alabama, AL, U.S.A.) and was purified further as described by Teien et al. [8]. Chondroitin-4-sulfate from bovine nasal cartilage and chondroitin-6-sulfate from shark cartilage were kindly supplied by Drs. A. Wasteson and T.C. Laurent, respectively (University of Uppsala, Uppsala, Sweden). Analytical data for the polysaccharide preparations are given in Table I.

Purification of chymotrypsin- and elastase-like enzymes from human granulocytes. Granules, obtained from granulocytes after differential centrifugation [10], were disrupted by freeze-thawing in liquid nitrogen, six times. After centrifugation the pellet was extracted [11] in 10 mM phosphate buffer, pH 7.4/100 mM NaCl (extract I), then in the same buffer containing 1.0 M NaCl (extract II).

Purification of the elastase-like enzyme was carried out according to Baugh and Travis [12]. Extract II was dialyzed in 50 mM Tris-HCl buffer, pH 8.0/50

TABLE I

ANALYSIS OF POLYSACCHARIDE PREPARATIONS

The analytical methods were as described by Jacobsson et al. [9]. Uronic acid and hexosamine values are expressed as percent of dry weight, not corrected for moisture and loss during hydrolysis.

Preparation	Uronic acid (%)	Hexosamine (%)	Sulfate * / Disaccharide
Heparin	23.5	29.0	2.33
Dermatan sulfate	23.4	28.9	1.18
Heparan sulfate	31.7	25.9	0.82
Chondroitin-4-sulfate	20.5	22.4	0.96
Chondroitin-6-sulfate	24.4	23.3	1.23

* Molar ratio with hexosamine as 1.00.

mM NaCl, whereupon the chymotrypsin-like enzyme precipitated. After centrifugation the supernatant was purified on BPTI-Sepharose. The elastase-like enzyme was eluted with 50 mM sodium acetate buffer, pH 5.0/1.0 M NaCl.

The chymotrypsin-like enzyme was also purified from extract II, according to Feinstein and Janoff [11] on a 4-phenylbutylamine Affi-gel 10. The enzyme was eluted with 10 mM phosphate buffer, pH 7.4/1.0 M NaCl/20% Me_2SO (v/v).

Both eluates, containing either elastase- or chymotrypsin-like enzyme proteins, were dialyzed against 10 mM phosphate buffer, pH 7.0/300 mM NaCl, then lyophilized.

Gel electrophoresis was performed in a 10% polyacrylamide gel at a current of 5 mA/tube [13]. The gel was stained with 1% Amido black 10B dissolved in 7% acetic acid.

Molecular weight was determined by gel electrophoresis [14] at a current of 8 mA/tube. A 10% polyacrylamide gel system was applied in the presence of 0.1% sodium dodecyl sulfate (SDS). The gel was stained with 2.5% Coomassie brillant blue R-250 dissolved in acetic acid/methanol mixture. BPTI (M_r 6500), lysozyme (14 600), soybean trypsin inhibitor (21 500), trypsin (24 000), chymotrypsin (25 000), ovalbumin (43 000) and bovine serum albumin (68 600) were used as reference proteins.

Titration for active centre of enzymes was carried out according to Winninger et al. [15] with human granulocyte cytosol inhibitor and BPTI.

The elastase-like enzyme (640 Boc-Ala-Np units/mg protein) was purified 6-fold, as calculated on the basis of the specific activity of the granule extract. The densitometric scan of the enzyme after gel electrophoresis showed three isoenzymes; their molecular weights were 30 300, 31 000 and 33 000. The active enzyme content was 93%.

The purification factor for the chymotrypsin-like enzyme (273 Bz-Tyr-OEt units/mg protein) was also 6-fold, as compared to the specific activity of the granule extract. The densitrometric scan of the enzyme after gel electrophoresis showed two isoenzymes; their molecular weights were 24 000 and 27 000. The active enzyme content was 81%.

Determination of esterolytic activity. The assays were performed in 50 mM

phosphate buffer, pH 7.6/5% Me_2SO, by determining the absorbances in a Zeiss (Oberkochen, F.R.G.) spectrophotometer equipped with a thermostated cell holder. For chymotrypsin Bz-Tyr-OEt in 500 μM final concentration and for elastase [16] Boc-Ala-Np in 260 μM final concentration were used as substrates, and the absorbances were recorded at 256 and 347.5 nm, respectively. $A_\lambda^{1cm} = 0.01$ absorbance change in 1 min at a given pH and 37°C was taken as unit esterolytic activity.

Determination of protein content. Protein concentration was determined by the method of Lowry et al. [17].

Glycosaminoglycan-coupled Sepharose 4B gels. The gels were prepared by mixing a polysaccharide, Sepharose 4B and CNBr under conditions described by Miller-Andersson et al. [18]. The glycosaminoglycan-coupled Sepharose gels were used for one experiment only, and were then discarded. The amount of polysaccharides bound per ml gel was determined as follows. The bound polysaccharides were hydrolyzed for 14 h with 4 N HCl in a boiling water bath, then evaporated and dissolved in distilled water, three times. The remainder was dissolved in water, bound on an 0.5 × 3.5 cm Dowex 50 × 8 ion-exchange column in H^+-form, washed three times with 1 ml distilled water and then eluted with 1.5 ml 2 N HCl. To remove HCl the eluate was evaporated, dissolved in distilled water and evaporation was then repeated twice. Finally, the remainder was dissolved in 0.5 ml 0.3 N HCl for determination of the hexosamine content by the Elson-Morgan reaction as modified by Gardell [19]. The polysaccharide content of the substituted gels was calculated from the resulting color yields, corrected for loss during the procedure; this correction was based on the recovery of hexosamine following similar analysis of the corresponding unbound polysaccharides.

Results

pH-Dependence of the chymotrypsin-like activity. When assayed at pH 5.0, the Bz-Tyr-OEt-splitting activity of the human granulocyte chymotrypsin-like enzyme is only about one-fourth of that measured at pH 7.6. When the enzyme (5 μg/ml) was preincubated for 5 min at 37°C with glycosaminoglycans (at a glycosaminoglycan/enzyme ratio of 3 : 1, w/w) in phosphate buffer, pH 5.0—7.6, a higher enzyme activity was detected than in the absence of glycosaminoglycans. Furthermore, the esterolytic activity of the mixture containing both the enzyme and a glycosaminoglycan was nearly independent of the changes in pH. The further experiments were, therefore, carried out at pH 6.0, which is appropriate to the pH of the human granulocyte lysosome [20].

Dependence of the esterolytic activity of the chymotrypsin-like enzyme on glycosaminoglycan concentration. Chymotrypsin-like enzyme (5 μg/ml) was preincubated for 5 min at 37°C with various glycosaminoglycans (at a glycosaminoglycan/enzyme ratio of 0—3 : 1, w/w) in phosphate buffer, pH 6.0. Enzyme activity was then determined with Bz-Tyr-OEt substrate (Fig. 1).

The inhibitions obtained for the chymotrypsin-like enzyme with various polysaccharides were rather moderate. Although heparin proved to be slightly more inhibitory than the other glycosaminoglycans, the remaining chymotrypsin-like activity measured after inhibition at a heparin/enzyme ratio of

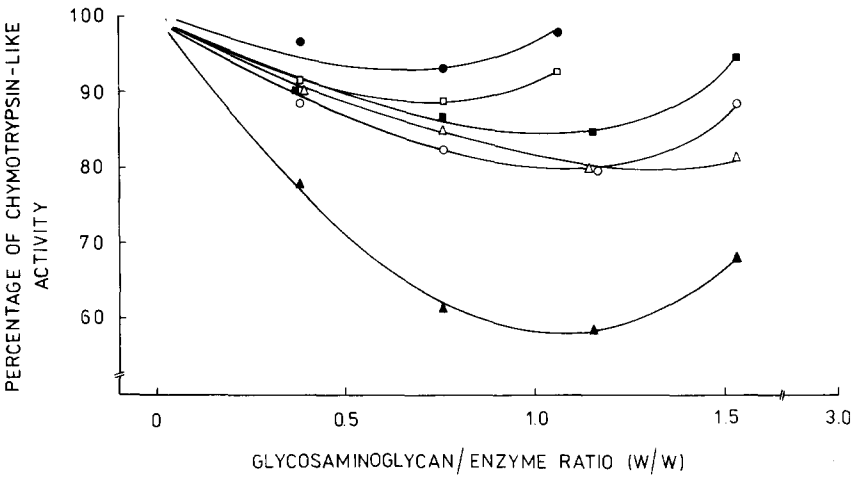

Fig. 1. Dependence of the esterolytic activity of the chymotrypsin-like enzyme of the human granulocyte on glycosaminoglycan concentration. The enzyme (5 μg/ml) was preincubated for 5 min at 37°C with glycosaminoglycans at a glycosaminoglycan/enzyme ratio of 0—3 : 1 (w/w) in phosphate buffer, pH 6.0. Enzyme activity was determined with Bz-Tyr-OEt substrate (500 μM final conc.) and was compared to the control (100% activity) obtained without glycosaminoglycans. Symbols: ▲———▲, chymotrypsin-like activity measured with heparin; ○———○, with heparan sulfate; △———△, with chondroitin-6-sulfate; ■———■, with dermatan sulfate; □———□, with hyaluronic acid; ●———●, with chondroitin-4-sulfate.

1.15 : 1 was still 60%. The interaction was in general heparin > chondroitin-6-sulfate, heparan sulfate, dermatan sulfate, chondroitin-4-sulfate, hyaluronic acid. The extent of effect depended upon the polysaccharide/enzyme protein weight ratio; at ratios above 2 : 1 the inhibitory effect of the polysaccharides was eliminated.

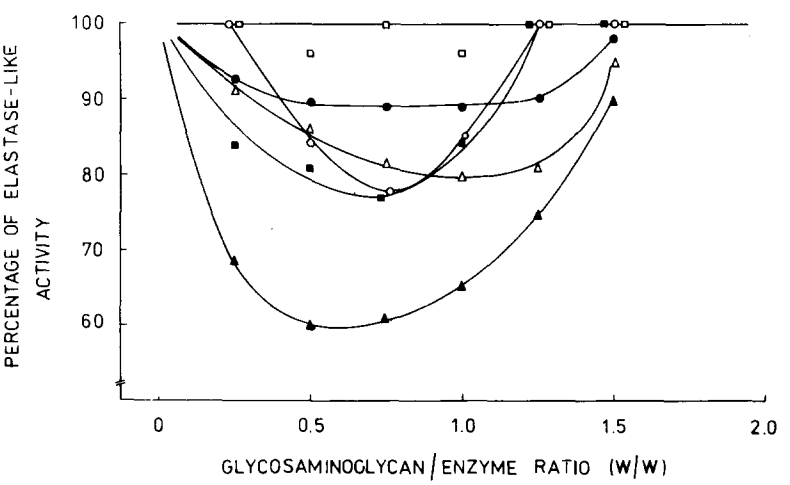

Fig. 2. Effect of glycosaminoglycan concentration on the esterolytic activity of the elastase-like enzyme of the human granulocyte. The enzyme (10 μg/ml) was preincubated for 5 min at 37°C with glycosaminoglycans at a glycosaminoglycan/enzyme ratio of 0—2 : 1 (w/w) in phosphate buffer, pH 6.0. Enzyme activity was determined with Boc-Ala-Np substrate (260 μM final conc.) and compared to the control (100% activity) obtained without glycosaminoglycans. Symbols: ▲———▲, elastase-like activity measured with heparin; ○———○, with heparan sulfate; △———△, with chondroitin-6-sulfate; ■———■, with dermatan sulfate; □———□, with hyaluronic acid; ●———●, with chondroitin-4-sulfate.

Effect of the glycosaminoglycan concentration on the esterolytic activity of the elastase-like enzyme. Human granulocyte elastase-like enzyme (10 μg/ml) was preincubated for 5 min at 37°C with various polysaccharides (at a glycosaminoglycan/enzyme ratio of 0—2 : 1, w/w) in phosphate buffer, pH 6.0. Enzyme activity was then assayed with Boc-Ala-Np substrate.

Fig. 2 shows that heparin had comparatively the strongest inhibitory effect (40%) on the esterolytic activity of the elastase-like enzyme at a heparin/enzyme ratio of 0.5 : 1. No inhibition could be detected with hyaluronic acid. The degree of the glycosaminoglycan-elastase interaction was in general heparin > heparan sulfate, dermatan sulfate, chondroitin-6-sulfate, chondroitin-4-sulfate > hyaluronic acid.

Binding of the chymotrypsin- and elastase-like enzymes on polysaccharide-Sepharose gels. The method of Bengtsson et al. [21] developed for the study of lipoprotein lipase was adapted to investigate further the interaction of human granulocyte proteases with glycosaminoglycans. Glycosaminoglycans were coupled to Sepharose 4B by direct activation with cyanogen bromide. The polysaccharide content of the affinity gels thus obtained is presented in Table II. 70 μg enzyme protein were incubated for 1 h at 4°C with 100 μl of the appropriate gel in an incubation mixture of 1-ml final colume (pH 6.0). After centrifugation the remaining activity of the supernatant was measured. The values obtained are summarized in Table II.

All the glycosaminoglycans, except hyaluronic acid, were capable of binding the elastase-like enzyme. Binding was the strongest on the heparin-Sepharose, and it was only slightly influenced within the range of experimental error by the amount of heparin (1.28 or 0.52 mg/ml) coupled to the gel. The degree of binding of the elastase-like enzyme to the polysaccharide gel was heparin >

TABLE II

BINDING OF THE CHYMOTRYPSIN- AND ELASTASE-LIKE ENZYMES OF THE HUMAN GRANULOCYTE TO POLYSACCHARIDE-SEPHAROSE GELS

The standard error of the estimate of mean value was 100 ± 10%. Bound polysaccharides were hydrolyzed with 4 N HCl and the liberated hexosamine was determined by the Elson-Morgan method. 70 μg enzyme were equilibrated with 100 μl of the respective gel in 50 mM phosphate buffer, pH 6.0/125 mM NaCl in 1-ml final volume. After agitation for 1 h at 4°C the suspensions were centrifuged at 2000 × *g* for 2 min. The enzyme activity of the supernatants (800 μl each) was determined in 100 mM phosphate buffer, pH 7.6. H, Heparin; DS, dermatan sulfate; HS, heparan sulfate; CSA-6S, chondroitin-6-sulfate; CSA-4S, chondroitin-4-sulfate; HA hyaluronic acid.

Preparation	Polysaccharide content (mg/ml)	Remaining activity	
		Elastase (%)	Chymotrypsin (%)
None	0.00	100	100
Sepharose 4B	0.00	no binding	
H-Sepharose 4B	1.28	15	45
	0.52	29	50
DS-Sepharose 4B	1.04	55	93
HS-Sepharose 4B	1.00	18	85
CSA-6S-Sepharose 4B	0.37	35	56
CSA-4S-Sepharose 4B	0.24	60	70
HA-Sepharose 4B	0.90	no binding	

TABLE III

BINDING OF THE CHYMOTRYPSIN- AND ELASTASE-LIKE ENZYMES OF THE HUMAN GRANULOCYTE ON POLYSACCHARIDE-SEPHAROSE GELS

The standard error of the estimate of mean value was 100 ± 10%. The enzymes were equilibrated with the respective gels at 4°C. After centrifugation 800 µl 50 mM phosphate buffer, pH 6.0/0.3, 0.6 or 1.2 M NaCl were successively applied to the pellets. The supernatants were assayed for enzyme activity and compared to the control (100% activity) measured in the absence of the gel. For abbreviations see Table I. All values are given as percentages. Elast., Elastase-like; Chym., chymotrypsin-like.

Eluent: 50 mM phosphate, pH 6.0	Activity of Enzymes measured in the Supernatant of											
	H-Sepharose		DS-Sepharose		HS-Sepharose		CSA-6S-Sepharose		CSA-4S-Sepharose		HA-Sepharose	
	Elast.	Chym.	Elast.	Chym.	Elast.	Chym.	Elast.	Chym.	Elast.	Chym.	Elast.	Chym.
0.125 M NaCl	15	45	55	93	18	85	35	56	60	70	100	100
0.300 M NaCl	15	—	30	—	13	—	50	—	38	30	—	—
0.600 M NaCl	52	52	18	7	52	15	16	46	—	—	—	—
1.200 M NaCl	9	6	—	—	11	—	—	—	—	—	—	—
Sum	91	103	103	100	94	100	101	102	98	100	100	100

heparin sulfate > chondroitin-6-sulfate > dermatan sulfate > chondroitin-4-sulfate > hyaluronic acid.

Compared to the elastase-like enzyme the binding of the chymotrypsin-like enzyme to the glycosaminoglycan gels was weaker. Further, the degree of binding found at a constant glycosaminoglycan/enzyme protein weight ratio varied in a different order: heparin > chondroitin-6-sulfate > chondroitin-4-sulfate > heparan sulfate > dermatan sulfate > hyaluronic acid.

Neither of these two enzymes could be bound to free, CNBr-activated Sepharose 4B, under the conditions applied in the present experiments.

When the enzymes bound to the gels were stepwise eluted with phosphate buffer containing 0.3, 0.6 or 1.2 M NaCl, the strongest binding was again seen with the heparin-Sepharose gel (Table III). For example, 52% of the elastase-like enzyme activity applied to the gel could be eluted with phosphate buffer containing 0.6 M NaCl and 9% with 1.2 M NaCl-containing buffer. Elution of the chymotrypsin-like enzyme gave similar results.

Binding of the enzyme was considerably weaker with chondroitin sulfate. From the chondroitin-4-sulfate-Sepharose gel both enzymes could be removed

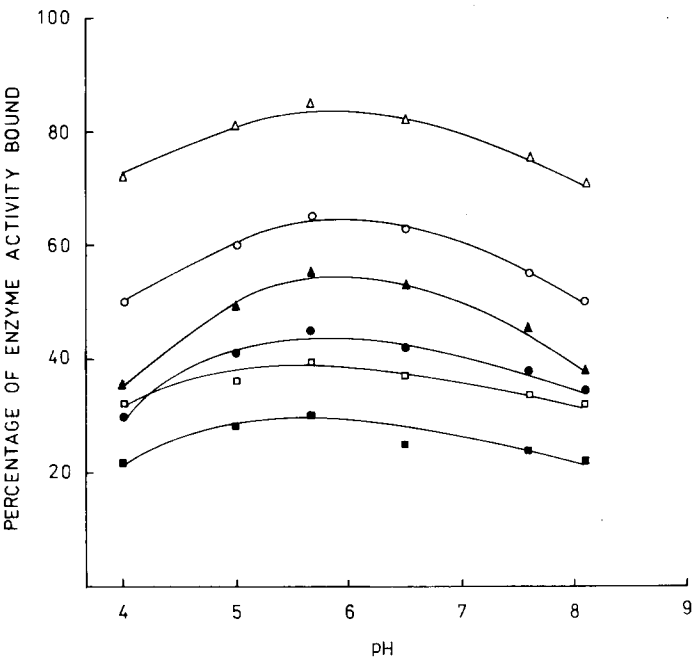

Fig. 3. Binding of the chymotrypsin- and elastase-like enzymes of the human granulocyte to polysaccharide-Sepharose gels as a function of pH. The enzymes (100 μg each) were equilibrated with 100 μl of the respective gel in 50 mM phosphate buffer, pH 5—8.2, or acetate buffer, pH 4.0, both containing 125 mM NaCl in 1-ml final volume. After 1 h agitation at 4°C the suspensions were centrifuged at 2000 × *g* for 2 min and the supernatants were removed for assay of enzyme activity. After washing the gels with 50 mM phosphate buffer, pH 6.0/125 mM NaCl, the enzymes were eluted with the above phosphate buffer containing 1.2 M NaCl. The eluates were assayed in 100 mM phosphate buffer, pH 7.6. The value of bound activities is expressed in percentage of the activity of the control enzyme (without gel) at a given pH. Symbols: △———△, bound elastase- and ▲———▲, bound chymotrypsin-like activity eluted from heparin-Sepharose 4B; □———□, bound elastase- and ■———■, bound chymotrypsin-like activity eluted from chondroitin-4-sulfate-Sepharose 4B; ○———○, bound elastase- and ●———●, bound chymotrypsin-like activity eluted from chondroitin-6-sulfate-Sepharose 4B.

at 0.3 M NaCl concentration. From chondroitin-6-sulfate-Sepharose the chymotrypsin-like enzyme could be eluted completely with 0.6 M NaCl whereas, a considerable percentage of the elastase-like activity could be removed with only 0.3 M NaCl. The three isoenzymes of the elastase-like enzyme eluted separately as well as the two isoenzymes of the chymotrypsin-like enzyme.

In preliminary experiments the protein content of extract II was bound on a glycosaminoglycan-Sepharose gel in a range of 10—100 μg. The shape of the adsorption isotherm was similar to that of the purified enzyme. Hence, protein-contaminants may not disturb the binding of the enzymes at this range.

The dependence on pH of the binding of the two enzymes was studied within the range of pH 4—8.2 using 50 mM sodium acetate/acetic acid buffer, pH 4.0, and 50 mM Na_2HPO_4/KH_2PO_4 buffer, pH 5.0—8.2. 100 μg enzyme were incubated for 1 h at 4°C with 100 μl polysaccharide-Sepharose 4B gel in the appropriate buffer in 1-ml final volume. After centrifugation the supernatant was assayed for enzyme activity. The gels were then washed with phosphate buffer, pH 6.0/125 mM NaCl. The bound enzymes were removed with 1.2 M NaCl containing buffer. The values of bound activity are presented as a function of pH in Figure 3. Within the range studied binding of the enzymes was found to be only slightly susceptible to pH, although, binding of both enzymes was somewhat more effective at pH 6.0.

Discussion

On the basis of earlier studies [22] the neutral proteases of granulocyte granules have been supposed to form an ionic interaction with the glycosaminoglycans. Further, this electrostatic interaction has been assumed to be responsible for the intracellular latency of enzymes [1].

In the present study glycosaminoglycans have been shown to bind the neutral proteases of the human granulocyte. The glycosaminoglycans entered into an ionic interaction with the elastase- and chymotrypsin-like enzymes, which are very basic proteins [23]. Heparin with the highest sulfate content, formed the most effective binding, whereas hyaluronic acid, without any sulfate group, was found to be ineffective. Apart from the sulfate content no apparent correlation between the structure of glycosaminoglycans and their interaction with neutral proteases was detected.

The glycosaminoglycan-enzyme interaction was found to manifest itself by moderate inhibition of the esterolytic activiy of the chymotrypsin- and elastase-like enzymes. In contrast with this, Avila and Convit [5] have reported that the acid hydrolases of the granules of human granulocytes are strongly inhibited by glycosaminoglycans; nevertheless, the elastase-like enzyme is only moderately inhibited. Baici et al. [24] have pointed out that the human granulocyte elastase-like enzyme is inhibited by the glycosaminoglycan polysulfate Arteparon®. Depending on the chain length of the polysulfated glycosaminoglycan, 2, 3 or 5 enzyme molecules can be tightly bound by a single inhibitor molecule. The excess of the glycosaminoglycan probably disturbs the arrangement of the enzyme along the chain.

Even this moderate inhibition of the proteolytic activity is eliminated at higher glycosaminoglycan/enzyme weight ratios. The results of our experiments

suggest that the interaction of neutral proteases with glycosaminoglycans depends on their ratio. A similar observation has been reported by Reggio and Dagorn [25] on the interaction between chymotrypsinogen and chondroitin sulfate.

The glycosaminoglycan/enzyme protein weight ratios investigated in the present experiments were selected to approximate the glycosaminoglycan/protein ratio found in the granulocyte granules [5,26].

Although the chymotrypsin-like enzyme has a higher isoelectric point than that of the elastase-like enzyme [23], it binds to heparin less strongly. This enzyme, however, forms stronger interactions with chondroitin-6-sulfate and chondroitin-4-sulfate than the elastase-like enzyme. Since the granulocyte granules contain mainly chondroitin-4-sulfate [2], this may account for the fact that, as compared to the elastase-like enzyme, the chymotrypsin-like enzyme is bound more effectively by chondroitin-4-sulfate and can be extracted from the granules with a buffer of higher ionic strength [11].

As regards the physiological role of the glycosaminoglycan enzyme interaction, the granulocyte granules contain chondroitin sulfates [2] which seem likely to interact with the neutral proteases in vivo. Nevertheless, the present results indicate that such an interaction will not significantly inhibit enzyme activity. This moderate inhibition can, therefore, not be responsible for the intracellular latency of enzymes. Instead, some uncertain sterical factors may be considered to be involved. It is conceivable that the catalytic site of the glycosaminoglycan-enzyme complex is inaccessible to the macromolecular, physiological substrates in vivo (contrary to the synthetic substrates). Moreover, a third component (a lipid or a protein-like protease inhibitor) may also be responsible for the inactive state of the proteases.

The glycosaminoglycan-enzyme interaction is also assumed to have a role in the attachment of these proteases to the lung, arterial wall and cell surface, all of which contain heparan sulfate and dermatan sulfate [27].

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