Interaction of lipoprotein lipase with native and modified heparin-like polysaccharides

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1. Lipoprotein lipase (EC 3.1.1.34), which was previously shown to bind to immobilized heparin, was now found to bind also to heparan sulphate and dermatan sulphate and to some extent to chondroitin sulphate. 2. The relative binding affinities were compared by determining (a) the concentration of NaCl required to release the enzyme from polysaccharide-substituted Sepharose; (b) the concentration of free polysaccharides required to displace the enzyme from immobilized polysaccharides; and (c) the total amounts of enzyme bound after saturation of immobilized polysaccharides. By each of these criteria heparin bound the enzyme most efficiently, followed by heparan sulphate and dermatan sulphate, which were more efficient than chondroitin sulphate. 3. Heparin fractions with high and low affinity for antithrombin, respectively, did not differ with regard to affinity for lipoprotein lipase. 4. Partially *N*-desulphated heparin (40–50% of *N*-unsubstituted glucosamine residues) was unable to displace lipoprotein lipase from immobilized heparin. This ability was restored by re-*N*-sulphation or by *N*-acetylation; the *N*-acetylated product was essentially devoid of anticoagulant activity. 5. Partial depolymerization of heparin led to a decrease in ability to displace lipoprotein lipase from heparin–Sepharose; however, even fragments of less than decasaccharide size showed definite enzyme-releasing activity. 6. Studies with hepatic lipase (purified from rat post-heparin plasma) gave results similar to those obtained with milk lipoprotein lipase. However, the interaction between the hepatic lipase and the glycosaminoglycans was weaker and was abolished at lower concentrations of NaCl. 7. The ability of the polysaccharides to release lipoprotein lipase to the circulating blood after intravenous injection into rats essentially conformed to their affinity for the enzyme as evaluated by the experiments *in vitro*.

While studying the anticoagulant effects of injected heparin, Hahn (1943) noted that heparin also caused the appearance in blood of a lipaemia ‘clearing factor’. Korn (1955) showed that this factor is a lipolytic enzyme and was able to recover enzyme preparations with similar properties from several tissues. The enzyme was called lipoprotein lipase because of its high activity against the triacylglycerol of plasma lipoproteins. It was later found that lipoprotein lipase binds to heparin and this binding has been exploited for affinity purification (Olivecrona *et al.*, 1971). Under certain conditions heparin can increase the activity of crude

enzyme preparations, but has no effect on purified lipoprotein lipase (Iverius *et al.*, 1972). More recently, a second lipase activity was demonstrated in post-heparin plasma; it probably originates in the liver and is usually referred to as hepatic lipase (La Rosa *et al.*, 1972). This lipase also binds to heparin (Hernell *et al.*, 1975). Although the ability to bind heparin seems to be a general property of these lipases (Olivecrona *et al.*, 1977) it is not known what molecule, if any, occupies the heparin-binding site *in vivo*. The present study demonstrates that the lipases bind also to other sulphated polysaccharides, such as heparan sulphate and dermatan sulphate. The

structural features required for binding of various glycosaminoglycans, particularly heparin, to lipoprotein lipase have been studied in some detail.

Experimental

Preparation and assay of lipases

Lipoprotein lipase was purified from bovine milk as described (Bengtsson & Olivecrona, 1977). Hepatic lipase was obtained from post-heparin plasma of rats injected 7 min earlier with 1 mg of heparin/kg body wt. It was partially purified by chromatography on heparin-Sepharose (Hernell *et al.*, 1975).

Lipase activities were measured as described (Hernell *et al.*, 1975) at pH 8.5 with [3 H]oleic acid-labelled trioleoylglycerol emulsified in gum arabic as substrate. Lipoprotein lipase was assayed in 0.1 M-NaCl with human serum added as source of activator; hepatic lipase was assayed in 1 M-NaCl in the absence of serum. One unit of lipase is defined as the amount of enzyme that releases one μ mol of fatty acid/min at 37°C.

Glycosaminoglycans

Analytical data for the polysaccharide preparations are given in Table 1. Heparin (stage 14) isolated from pig intestinal mucosa was purchased from Inolex Pharmaceutical Division, Park Forest South, IL, U.S.A. and was purified by precipitation with cetylpyridinium chloride from 1.2 M-NaCl (Lindahl *et al.*, 1965).

Fractionation of heparin with regard to its affinity for antithrombin was carried out by affinity chromatography on antithrombin-Sepharose (Laurent *et al.*, 1978).

Partial *N*-desulphation of heparin was achieved by treating the polysaccharide with 0.04 M-HCl at 50°C for varying periods of time (Riesenfeld *et al.*, 1977). In some experiments the amino groups exposed during previous *N*-desulphation were acetylated by treatment with acetic anhydride (Danishefsky & Steiner, 1965) or resulphated by treatment with trimethylamine- SO_3 complex (Levy & Petracek, 1962).

To prepare heparin fragments differing with regard to molecular size, 50 mg of heparin was treated with nitrous acid (reaction A in Lindahl *et al.*, 1973) for 8 min at room temperature (20°C). The degraded polysaccharide was fractionated on a column (2 cm $\times$ 75 cm) of Sephadex G-75 in 0.5 M- NH_4HCO_3 and the effluent fractions were pooled in six fractions (I–VI) and freeze-dried. The molecular size of the material in fractions I–IV was estimated by gel chromatography on a column (1 cm $\times$ 145 cm) of Sephadex G-50, equilibrated with 1 M-NaCl. Comparison with reference oligosaccharides (kindly donated by Dr. T. C. Laurent, University of

Uppsala, Sweden) obtained by digestion of [3 H]acetyl-labelled hyaluronic acid with testis hyaluronidase, indicated the following mean number of saccharide units/molecule; fraction I, 14; fraction II, 12; fraction III, 8; fraction IV, 6. The molecular weights of fractions I–III, also determined by sedimentation equilibrium ultracentrifugation (Jansson *et al.*, 1975), were 3800, 3000 and 2100, respectively, in fair agreement with the values obtained by gel chromatography (assuming a molecular weight for a heparin disaccharide unit of about 600).

Heparan sulphate was prepared from human aorta, as described by Iverius (1971). Analysis of the final product by ion-exchange chromatography showed a low-sulphated material without any detectable heparin-like components.

Dermatan sulphate, prepared by alkaline copper precipitation from heparin by-products, was a gift from Mrs. Kajsa Rodén, University of Alabama, Birmingham, AL, U.S.A., and was further purified as described (Teien *et al.*, 1976).

Chondroitin 4-sulphate from bovine nasal cartilage was kindly donated by Dr. Å. Wasteson, University of Uppsala, Sweden.

Glycosaminoglycan-substituted agarose gels

Most gels were prepared according to Iverius (1971); 2 mg of polysaccharide was added/ml of CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden). Heparin-Sepharose gels with a lower degree of substitution (designated 1/10 and 1/50 heparin-Sepharose) were prepared by adding 0.2 mg and 0.04 mg of heparin/ml of activated Sepharose, respectively. Some substituted gels were prepared by mixing polysaccharide, non-activated Sepharose 4B and CNBr under the conditions described by Miller-Andersson *et al.* (1974). Glycosaminoglycan-substituted Sepharose gels were used for one experiment only and were then discarded. The amounts of polysaccharide bound/ml of gel are indicated in the legends to the Figures.

Studies of enzyme-glycosaminoglycan interaction in vitro

Equilibration experiments. An enzyme solution was equilibrated with a small volume of polysaccharide-substituted gel for at least 30 min under the conditions described in detail in the legend to Table 2. Bovine serum albumin (1%, w/v) was added to increase the stability of the enzyme and to decrease non-specific binding to the gel (Bengtsson & Olivecrona, 1977). After centrifugation a sample of the supernatant was taken for assay of enzyme activity. To evaluate the effect of salts or of free, competitive, polysaccharides on the binding of enzyme to the immobilized polysaccharides, sequential additions were made at 30 min intervals and the

Table 1. *Analysis of polysaccharide preparations*

The analytical methods used were as described by Jacobsson *et al.* (1979). Uronic acid and hexosamine values are expressed as percentage of dry wt., not corrected for moisture nor for losses during hydrolysis. The ratio glucosamine/total hexosamine was 1.0 for heparin and heparan sulphate and 0.0 for chondroitin sulphate and dermatan sulphate. The L-iduronic acid and sulphate contents are expressed as molar ratios, with total uronic acid and hexosamine, respectively, as 1.0. The N-substitution pattern of heparin-like polysaccharides was determined by gel chromatography of the products obtained by selective deamination with nitrous acid (Jacobsson *et al.*, 1979); the results are expressed as percentage of total hexosamine units. Anti-coagulant activities were determined according to the British Pharmacopoeia (1968).

Preparation	Uronic acid (%)	Hexosamine (%)	L-Iduronic acid/ D-glucuronic acid) (molar ratio)	Sulphate/ hexosamine (molar ratio)	Glucosamine residues (%) with			Anticoagulant activity (units/mg)
					Unsubstituted amino groups	Sulphated amino groups	Acetylated amino groups	
Heparin	24	29	0.67	2.3	<5	70	30	165
Heparin, treated with 0.04 M-HCl for 16 h	27	22		2.1	40	35	25	10
Heparin, treated with 0.04 M-HCl for 16 h and N-acetylated	25	23		1.9	<5	30	70	<10
Heparin, fraction with high affinity for antithrombin	26	30		2.2	<5	70	30	257
Heparin, fraction with low affinity for antithrombin	26	24		2.3	<5	75	25	11
Heparan sulphate	29	35	0.15	0.5	<5	35	60	<10
Chondroitin sulphate	27	30	<0.05*	0.6				<10
Dermatan sulphate	23	29	<0.85*	1.2				<10

* Determined by gel chromatography of the products obtained after digestions of the polysaccharides with chondroitinase AC (Malmström *et al.*, 1975).

enzyme activity in the supernatant was assayed at the end of each interval.

Saturation experiments. Enzyme solution [35 units/ml in 5 mM-sodium veronal buffer, pH 7.4, containing 0.3 M-NaCl and 1% (w/v) bovine serum albumin] was passed through small columns (0.1 ml) of polysaccharide-substituted Sepharose (0.1–0.3 mg of ligand/ml of gel). After passage of a total of 440 units of lipoprotein lipase through the columns the enzyme activity in the effluents exceeded 90% of that of the solutions applied. The columns were then eluted with 10 ml of 1.5 M-NaCl in the same buffer, and the enzyme activity of the eluate was determined.

Experiments in vivo

To assess the ability of the native or modified glycosaminoglycans to release lipases *in vivo*, the polysaccharides were injected intravenously into rats. After 7 min, blood was withdrawn from the exposed abdominal aorta. Plasma was prepared and was analysed for lipoprotein lipase and for hepatic lipase, either directly or after separation of the two enzymes by chromatography on heparin–Sepharose (Bengtsson *et al.*, 1977).

Results

Interaction in vitro between lipoprotein lipase and glycosaminoglycans

Lipoprotein lipase was virtually quantitatively adsorbed to heparin–Sepharose at 0.32 M-NaCl. On increasing the ionic strength of the incubation medium, no significant release of enzyme occurred at lower concentrations of NaCl than 0.6 M; complete liberation of the enzyme required about 1.0 M-NaCl. At 0.88 M-NaCl 50% of the enzyme was in solution (Table 2). Both heparan sulphate–Sepharose and dermatan sulphate–Sepharose bound more than 85% of the enzyme at 0.32 M-NaCl, but the enzyme was released at lower salt concentrations from these gels than from the heparin–Sepharose (Table 2). The chondroitin sulphate–Sepharose bound less than 50% of the enzyme at 0.32 M-NaCl (Table 2); furthermore the adsorbed enzyme was released by a very moderate increase in ionic strength. No experiments were carried out at lower concentrations of NaCl than 0.32 M, owing to the limited stability and solubility of the enzyme under these conditions.

Table 3 summarizes experiments in which free and immobilized polysaccharide molecules were allowed to compete for the lipoprotein lipase

Table 2. *Displacement of lipases from glycosaminoglycan-substituted Sepharose on addition of NaCl to the liquid phase*
Enzyme (about 12 µg of lipoprotein lipase or equivalent activity of the partially purified hepatic lipase) was equilibrated with 0.1 ml of the respective gels in a total volume of 1 ml of 5 mM-sodium veronal buffer, pH 7.4, containing 0.32 M-NaCl and 1% bovine serum albumin. After 1 h at 4°C with gentle agitation the tubes were centrifuged at 2000 g for 2 min and samples of the supernatants were removed for assay of enzyme activity. After appropriate additions of 5 M-NaCl the contents of the tubes were mixed and incubated for 20 min as above. Centrifugation, sampling of the supernatants and further additions of NaCl (stepwise concentration increments of 0.1 M up to 1.0 M-NaCl) were repeated in the same manner, such that all data for one type of gel were obtained from the same tube. The enzyme activity in the liquid phase was plotted versus salt concentration (see Figs. 2 and 3 in Bengtsson & Olivecrona, 1977) and the concentration of NaCl corresponding to 50% of the lipase in the liquid phase was estimated by interpolation.

Enzyme	Polysaccharide ligand	Ligand concentration (mg/ml of gel)	Concn. of NaCl corresponding to 50% of the lipase activity in the liquid phase (M)
Lipoprotein lipase	Heparin	0.09	0.88
	Heparan sulphate	0.27	0.57
	Dermatan sulphate	0.08	0.46
	Chondroitin sulphate	0.09	0.32
	Heparin with high affinity for antithrombin	0.10	0.90
	Heparin with low affinity for antithrombin	0.12	0.86
	Partially <i>N</i> -desulphated and then <i>N</i> -acetylated heparin	0.15	0.73
Hepatic lipase	Heparin	0.09	0.45
	Heparan sulphate	0.27	0.21
	Dermatan sulphate	0.08	0.20
	Chondroitin sulphate	0.09	No measurable binding

Table 3. *Displacement of lipoprotein lipase from glycosaminoglycan-substituted Sepharose on addition of glycosaminoglycans to the liquid phase*

Conditions were as in Table 2, but with sequential additions of glycosaminoglycans instead of salt. These gave the following concentrations in the liquid phase: 20, 70, 170, 270, 370, 470 $\mu\text{g/ml}$ for heparan sulphate and for dermatan sulphate, and 0.21, 0.7, 1.7, 2.7, 3.7 and 4.7 $\mu\text{g/ml}$ for heparin. The polysaccharide-substituted gels were the same as in Table 2. The Table gives the estimated glycosaminoglycan concentrations corresponding to 50% of the enzyme activity in the liquid phase.

Seph- arose-bound ligand	Free ligand	Concentration of free ligand corresponding to 50% of the lipase activity in the liquid phase ($\mu\text{g/ml}$)		
		Heparan sulphate	Dermatan sulphate	Heparin
Heparan sulphate		70	135	1.8
Dermatan sulphate		20	20	1.3

molecules. Heparin at low concentration, and heparan sulphate or dermatan sulphate at higher concentrations, displaced the enzyme from heparan sulphate- or dermatan sulphate-substituted Sepharose. Chondroitin sulphate was less efficient than heparan sulphate or dermatan sulphate; less than 8% of the enzyme was released at 200 $\mu\text{g/ml}$ and even 2200 μg of chondroitin sulphate/ml released only 42 and 37% of the enzyme from heparan sulphate-Sepharose and dermatan sulphate-Sepharose, respectively. Heparan sulphate, dermatan sulphate and chondroitin sulphate were unable to release lipoprotein lipase from heparin-Sepharose at any concentration tested. Taken together with the results of the salt displacement experiments described above, these observations indicate that the affinity of glycosaminoglycans for lipoprotein lipase decreases in the order heparin > heparan sulphate $\approx$ dermatan sulphate > chondroitin sulphate.

The enzyme-binding ability of the preparations of heparan sulphate and dermatan sulphate could conceivably reflect the presence of heparin contaminants in these preparations. The analytical data indicated that such contaminants, if any, were present in very small amounts (Table 1). If heparin contaminants accounted for the lipoprotein lipase-binding properties of the heparan sulphate and dermatan sulphate preparations, the total enzyme-binding capacity of these preparations should be very low. Attempts to determine this capacity for various polysaccharide-substituted gels, by equilibrium experiments over a wide range of enzyme concentrations, were not feasible due to the restricted solubility properties of the lipoprotein lipase. Instead, the total amounts of enzyme bound to the gels at near-saturation were determined, as described in the Experimental section. Although such experiments will not directly define the actual number of enzyme-binding sites/polysaccharide chain (Bengtsson & Olivecrona, 1977) the results should serve to indicate the relative binding capa-

city of different polysaccharides. Using the same polysaccharide-Sepharose gels as in the equilibrium experiments, the enzyme-binding capacity of the heparan sulphate- and dermatan sulphate-substituted gels at 0.3 M-NaCl was 30% and 70% respectively of that of the heparin-substituted gel, on a polysaccharide-weight basis. These results clearly show that heparan sulphate and dermatan sulphate have enzyme-binding capacities of the same order of magnitude as that of heparin. Heparan sulphate and dermatan sulphate thus provide numerous, but relatively weak, binding sites for lipoprotein lipase.

Structural requirements for binding of heparin to lipoprotein lipase

Role of the *N*-substituent. Heparin was partially *N*-desulphated by treatment with 0.04 M-HCl at 50°C for various periods of time. The material obtained after 8 h of hydrolysis showed a low but significant ability to displace lipoprotein lipase from immobilized heparin, but after 16 h of hydrolysis this ability was completely lost (Fig. 1). The latter sample retained about half of the initial *N*-sulphate groups (Table 1). After re-*N*-sulphation this material displaced lipoprotein lipase as efficiently as did the starting material. However, also *N*-acetylation restored full ability to release the enzyme (Fig. 1). Saturation experiments showed no significant difference in enzyme-binding capacity between gels substituted with the partially *N*-desulphated, *N*-acetylated heparin and with the heparin starting material, respectively. However, a somewhat lower salt concentration was required to displace the enzyme from the modified material than from the native heparin (Table 2). This may reflect a lower affinity of the modified heparin for the enzyme.

Role of molecular size. Heparin fragments of various molecular sizes were added to samples of lipoprotein lipase, bound to unmodified, immobilized heparin (Fig. 2). The ability of the fragments to displace the enzyme decreased continuously with

Fig. 1. Displacement of lipoprotein lipase from heparin-Sepharose by partially *N*-desulphated heparin before and after *N*-acetylation or re-*N*-sulphation

The conditions were as described in Table 2, but a heparin-Sepharose with a lower degree of substitution was used [1/50 heparin-Sepharose (see the Experimental section)]. Addition of unmodified heparin (●), heparin hydrolysed with 0.04 M-HCl at 50°C for 0 h (○), 8 h (▲), and 16 h (■), heparin hydrolysed for 16 h and then re-*N*-sulphated (▼) and heparin hydrolysed for 16 h and then *N*-acetylated (□) is shown. ▽, Control with no gel and no additions. Altogether 100 μl was added and 35 μl was withdrawn from each tube.

Fig. 2. Displacement of lipoprotein lipase from heparin-Sepharose by heparin fragments of different sizes

Conditions were as described in Table 2. Heparin was depolymerized by partial deamination and the products were fractionated by gel filtration and are designated I-VI according to their order of elution from the column. I (▲), II (△), III (■), IV (□), V (▼), VI (▽) and unmodified heparin (●) were added. ○, Control with no gel, additions of unmodified heparin. Altogether 67 μl was added and 30 μl was withdrawn from each tube.

decreasing molecular size. Whereas the smallest components tested (fractions V and VI) were essentially inactive, fragments of hexa- or octasaccharide size (fractions III and IV) showed definite, albeit low, enzyme-releasing activity. This activity was further increased for the larger fractions (I and II), containing fragments of up to about tetradecasaccharide size. However, even the largest fragments were less active than the intact heparin molecule; thus 30 μg of fraction I or at least 50 μg of fraction II were required to equal the enzyme-releasing capacity of 2 μg of heparin.

Relation to heparin-antithrombin interaction. Separation of heparin by affinity chromatography on matrix-bound antithrombin yields two distinct fractions, one with low affinity for antithrombin and very low anticoagulant activity, and one with high affinity for antithrombin and high anticoagulant activity (see Lindahl *et al.*, 1979, for references). The two fractions could not be distinguished by chromatography on immobilized lipoprotein lipase, and showed equal ability to displace lipoprotein lipase from immobilized (unfractionated) heparin (Bengtsson *et al.*, 1977). These results suggested that the heparin molecule contains different binding sites for antithrombin and for lipoprotein lipase. This conclusion is supported by the results obtained in the present study, showing that the same NaCl concentration is required to displace lipoprotein lipase from immobilized heparins having high and low affinity for antithrombin, respectively (Table 2).

Interaction in vitro between hepatic (heparin-releasable) lipase and glycosaminoglycans

Experiments analogous to those described above for lipoprotein lipase were also carried out with hepatic lipase partially purified from rat post-heparin plasma. In equilibration experiments this lipase bound to Sepharose gels substituted with either heparin, heparin fractions separated with regard to affinity for antithrombin, partially *N*-desulphated and *N*-acetylated heparin or heparan sulphate. The various polysaccharides showed the same relative affinities for hepatic lipase as for lipoprotein lipase, as judged from salt-displacement experiments (Table 2). However, the salt concentration required to displace the hepatic lipase was always lower than for lipoprotein lipase, suggesting a lower affinity of the hepatic lipase for these polysaccharides. The difference was quite large; for instance, 50% displacement of enzyme activity from a heparin-substituted Sepharose gel occurred at 0.45 M-NaCl for the hepatic lipase but at 0.88 M-NaCl for lipoprotein lipase. As with lipoprotein lipase (Bengtsson & Olivecrona, 1977) the salt-displacement properties of the hepatic lipase depended on the nature of the salt and on the degree of polysaccharide substitution of the gel.

Table 4. Release of lipases into the circulating blood by intravenous injection of polysaccharide preparations
The determination of salt-resistant lipase (considered to represent hepatic lipase) and serum-stimulated lipase (lipoprotein lipase) was as described in a previous paper (Bengtsson *et al.*, 1977).

Injected preparation	Amount (mg/kg body wt.)	Enzyme activity (c.p.m. of ³ H/ μ l of plasma)	
		Salt-resistant lipase	Serum-stimulated lipase
No injection (control)		17	12
Heparin	1.5	913	884
Heparin, treated with 0.04 M-HCl for 16 h	1.5	78	61
Heparin, treated with 0.04 M-HCl for 16 h and N-acetylated	1.5	520	580
Heparan sulphate	3	86	27
Dermatan sulphate	3	45	33
Chondroitin sulphate	3	13	13

The displacement of hepatic lipase from heparin- or heparan sulphate-substituted Sepharose on the addition of various competing polysaccharides to the liquid phase was also studied. The results of these experiments were qualitatively similar to those obtained with lipoprotein lipase. Thus, heparin subfractions with high affinity and low affinity for antithrombin, respectively, were equally efficient in displacing the hepatic lipase. Furthermore, the enzyme-displacing ability was lost by partial *N*-desulphation of heparin (16h hydrolysis) and was restored either by re-*N*-sulphation or by *N*-acetylation of the polysaccharide.

Studies in vivo

The ability to release lipoprotein lipase and hepatic lipase from their tissue sites to the circulating blood was tested by intravenous administration of the various glycosaminoglycans to rats. About 0.5 mg of heparin/kg body weight is required to induce half-maximal release of either enzyme (Bengtsson *et al.*, 1977). When other polysaccharides were injected in amounts of 1.5 or 3 mg, a comparable release of lipases occurred only with the partially *N*-desulphated and then *N*-acetylated heparin (Table 4). The partially *N*-desulphated heparin (16h hydrolysis), the heparan sulphate and the dermatan sulphate caused a small but significant release of both lipases, whereas chondroitin sulphate was essentially inactive. No attempt was made to rank the relatively inactive polysaccharides by injecting larger amounts of these materials.

Discussion

Previous studies have established that heparin binds strongly and reversibly to lipoprotein lipase (Bengtsson & Olivecrona, 1977). The present study

demonstrates that heparin is not the only polysaccharide that can bind lipoprotein lipase; this property is shared also by heparan sulphate and dermatan sulphate, and to a lesser extent, by chondroitin sulphate. However, in all experimental systems tested, the interaction of lipoprotein lipase with these polysaccharides was weaker than that with heparin. No attempt was made to differentiate between heparan sulphate and dermatan sulphate with regard to enzyme affinity; in fact, such attempts appear less meaningful in view of the extensive structural variability of these species (Lindahl & Höök, 1978).

Some observations regarding the structural features of polysaccharides that bind to lipoprotein lipase may be considered. Heparin, heparan sulphate and dermatan sulphate all have higher affinities for lipoprotein lipase than has chondroitin sulphate. This difference in affinity cannot simply be ascribed to the degree of polyanion character, as the heparan sulphate preparation used had a lower sulphate content and yet a higher affinity for the enzyme than had the chondroitin sulphate. The common structural denominator for the three effective polysaccharides is the presence of L-iduronic acid (which is not present in chondroitin sulphate); it therefore seems likely that this component is somehow important for the binding of polysaccharides to lipoprotein lipase (see Lindahl & Höök, 1978, for similar conclusions regarding interactions with other proteins, such as collagen and platelet factor 4). The hexosamine units should be *N*-substituted, since partial *N*-desulphation led to loss of enzyme-binding ability; apparently, free amino groups impede the interaction. However, the exact nature of this requirement is unclear. *N*-Sulphate groups are obviously not essential for enzyme binding, as seen from the binding properties of

dermatan sulphate (which contains exclusively *N*-acetylated hexosamine residues). Furthermore, a major fraction of the *N*-sulphate groups in heparin could be replaced by *N*-acetyl groups without appreciable loss of enzyme affinity. On the other hand, it is not known to what extent the remaining *N*-sulphate groups contributed to the high enzyme affinity of the heparin derivative. In a more recent experiment, *N*-acetylation of a completely *N*-desulphated heparin (obtained by treatment with aqueous dimethylsulphoxide; Inoue & Nagasawa, 1976) failed to restore the affinity for lipoprotein lipase (G. Bengtsson, T. Olivecrona, M. Höök, J. Riesenfeld & U. Lindahl, unpublished work). To elucidate fully the role of *N*-sulphate groups, graded *N*-desulphation should be performed, yielding a series of heparin derivatives with continuously varied *N*-sulphate/*N*-acetyl ratios.

The hepatic lipase has many properties in common with lipoprotein lipase. It hydrolyses the same substrates (Ehnholm *et al.*, 1974, 1975), shows the same positional specificity in hydrolysis of triacylglycerols (Åkesson *et al.*, 1976), and has a similar monomer molecular size (Östlund-Lindqvist & Boberg, 1977). Kinetic differences can be used to differentiate the two enzymes; in contrast with lipoprotein lipase, the activity of the hepatic lipase is not stimulated by apolipoprotein CII and is not strongly inhibited by 1 M-NaCl (La Rosa *et al.*, 1972; Greten *et al.*, 1972). The hepatic lipase, previously known to bind to heparin-Sepharose (Greten *et al.*, 1972; Hernell *et al.*, 1975), was now found to bind to the same modified heparins and other polysaccharides as did lipoprotein lipase. However, the binding was always weaker with the hepatic lipase, indicating a lower affinity; the hepatic lipase was thus displaced from gel-bound polysaccharides at lower salt concentrations than was lipoprotein lipase. In all experimental systems tested, the various polysaccharides showed essentially the same relative affinities for hepatic lipase as for lipoprotein lipase. Although the binding of hepatic lipase is weaker than that of lipoprotein lipase, these results suggest that the same requirements regarding polysaccharide structure apply to the binding of either enzyme. It is of interest to note that whereas the hepatic lipase is monomeric (Ehnholm *et al.*, 1974), the lipoprotein lipase used here is dimeric (Iverius & Östlund-Lindqvist, 1976); the presence of two co-operating binding sites in lipoprotein lipase could explain the higher affinity.

It is of interest to compare binding of glycosaminoglycans to lipoprotein lipase (or hepatic lipase) with that to antithrombin. As discussed above, the interaction with the lipase appears to be relatively nonspecific; accordingly, polyanions other than polysaccharides, such as polynucleotides, appear capable of binding to lipoprotein lipase (Olivecrona

& Bengtsson, 1978). With antithrombin the situation is different, since binding of heparin to this protein depends strictly on the presence of variously substituted monosaccharide units, without any apparent relation to charge density (Lindahl *et al.*, 1979). Heparin molecules that lack this particular binding sequence (and thus have low affinity for antithrombin) still interact strongly with lipoprotein lipase. There is no indication of the involvement of any similar or analogous binding site in the heparin-lipase interaction. This difference is clearly illustrated by the effects of *N*-desulphation. Partial *N*-desulphation of heparin leads to partial loss of anticoagulant activity that cannot be restored by acetylation of the exposed free amino groups (Nagasawa *et al.*, 1977), and recent studies indicate a stringent requirement for *N*-sulphate groups in certain positions of the antithrombin-binding sequence (J. Riesenfeld, M. Höök & U. Lindahl, unpublished work). In contrast, lipase binding was abolished by partial *N*-desulphation but was then restored by subsequent *N*-acetylation. As expected, the resulting modified polysaccharide was essentially devoid of anticoagulant activity (Table 1). It is thus possible to prepare heparin derivatives that have low affinity for antithrombin but high affinity for lipoprotein lipase. Such preparations have been successfully used as affinity matrices for the chromatographic separation of lipoprotein lipase and antithrombin (G. Bengtsson & T. Olivecrona, unpublished work).

Chromatography of heparin on immobilized lipoprotein lipase affords subfractions that differ with regard to affinity for this enzyme (Olivecrona & Bengtsson, 1978). The affinity of these subfractions for antithrombin has not been studied. A possibility that remains to be explored is the preparation of heparin fractions (or derivatives) with high affinity for antithrombin and low affinity for the lipases.

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References

- Åkesson, B., Gronowitz, S. & Herslöf, B. (1976) *FEBS Lett.* **71**, 241–244
- Bengtsson, G. & Olivecrona, T. (1977) *Biochem. J.* **167**, 109–119
- Bengtsson, G., Olivecrona, T., Höök, M. & Lindahl, U. (1977) *FEBS Lett.* **79**, 59–63
- Brit. Pharmacopoeia* (1968) p. 1345, Pharmaceutical Press, London
- Danishefsky, I. & Steiner, H. (1965) *Biochim. Biophys. Acta* **101**, 37–45

- Ehnholm, C., Greten, H., & Brown, W. V. (1974) *Biochim. Biophys. Acta* **360**, 68–77
- Ehnholm, C., Shaw, W., Greten, H. & Brown, W. V. (1975) *J. Biol. Chem.* **250**, 6756–6761
- Greten, H., Walter, B. & Brown, W. V. (1972) *FEBS Lett.* **27**, 306–310
- Hahn, P. F. (1943) *Science* **98**, 19–20
- Hernell, O., Egelrud, T. & Olivecrona, T. (1975) *Biochim. Biophys. Acta* **381**, 233–241
- Inoue, Y. & Nagasawa, K. (1976) *Carbohydr. Res.* **46**, 87–95
- Iverius, P.-H. (1971) *Biochem. J.* **124**, 677–683
- Iverius, P.-H. & Östlund-Lindqvist, A.-M. (1976) *J. Biol. Chem.* **251**, 7791–7795
- Iverius, P.-H., Lindahl, U., Egelrud, T. & Olivecrona, T. (1972) *J. Biol. Chem.* **247**, 6610–6617
- Jacobsson, I., Bäckström, G., Höök, M., Lindahl, U., Feingold, D. S., Malmström, A. & Rodén, L. (1979) *J. Biol. Chem.* **254**, 2975–2982
- Jansson, L., Ögren, S. & Lindahl, U. (1975) *Biochem. J.* **145**, 53–62
- Korn, E. D. (1955) *J. Biol. Chem.* **215**, 1–14
- La Rosa, J. C., Levy, R. I., Windmueller, G. & Fredrickson, D. S. (1972) *J. Lipid Res.* **13**, 356–363
- Laurent, T. C., Tengblad, A., Thunberg, L., Höök, M. & Lindahl, U. (1978) *Biochem. J.* **175**, 691–701
- Levy, L. & Petracek, F. J. (1962) *Proc. Soc. Exp. Biol. Med.* **109**, 901–905
- Lindahl, U. & Höök, M. (1978) *Annu. Rev. Biochem.* **47**, 385–417
- Lindahl, U., Cifonelli, J. A., Lindahl, B. & Rodén, L. (1965) *J. Biol. Chem.* **240**, 2817–2820
- Lindahl, U., Bäckström, G., Jansson, L. & Hallén, A. (1973) *J. Biol. Chem.* **248**, 7234–7241
- Lindahl, U., Bäckström, G., Höök, M., Thunberg, L., Franson, L.-Å. & Linker, A. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3198–3202
- Malmström, A., Carlstedt, I., Åberg, L. & Fransson, L.-Å. (1975) *Biochem. J.* **151**, 477–489
- Miller-Andersson, M., Borg, H., & Andersson, L.-O. (1974) *Thromb. Res.* **5**, 439–452
- Nagasawa, K., Tokuyasu, T. & Inoue, Y. (1977) *J. Biochem. (Tokyo)* **81**, 989–993
- Olivecrona, T. & Bengtsson, G. (1978) in *Int. Conf. Atherosclerosis Milan 1977* (Carlson, L. A., Paoletti, R., Sirtori, C. R. & Weber, G., eds.), pp. 153–157, Raven Press, New York
- Olivecrona, T., Egelrud, T., Iverius, P.-H. & Lindahl, U. (1971) *Biochem. Biophys. Res. Commun.* **43**, 524–529
- Olivecrona, T., Bengtsson, G., Marklund, S.-E., Lindahl, U. & Höök, M. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 60–65
- Östlund-Lindqvist, A.-M. & Boberg, J. (1977) *FEBS Lett.* **83**, 231–236
- Riesenfeld, J., Höök, M., Björck, I., Lindahl, U. & Ajaxon, B. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 39–43
- Teien, A., Abildgaard, K. & Höök, M. (1976) *Thromb. Res.* **8**, 859–867