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Anticoagulant activities of heparin oligosaccharides and their neutralization by platelet factor 4

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Oligosaccharides of well-defined molecular size were prepared from heparin by nitrous acid depolymerization, affinity chromatography on immobilized antithrombin III (see footnote on Nomenclature) and gel chromatography on Sephadex G-50. High affinity (for antithrombin III) octa-, deca-, dodeca-, tetradeca-, hexadeca- and octadeca-saccharides were prepared, as well as oligosaccharides of larger size than octadecasaccharide. The inhibition of Factor Xa by antithrombin III was greatly accelerated by all of these oligosaccharides, the specific anti-Factor Xa activity being invariably greater than 1300 units/μmol. The anti-Factor Xa activity of the decasaccharide was not significantly decreased in the presence of platelet factor 4, even at high platelet factor 4/oligosaccharide ratios. Measurable but incomplete neutralization of the anti-Factor Xa activities of the tetradeca- and hexadeca-saccharides was observed, and complete neutralization of octadeca- and larger oligo-saccharides was achieved with excess platelet factor 4. The octa-, deca-, dodeca-, tetradeca- and hexadeca-saccharides had negligible effect on the inhibition of thrombin by antithrombin III, whereas specific anti-thrombin activity was expressed by the octadecasaccharide and by the larger oligosaccharides. An octadecasaccharide is therefore the smallest heparin fragment (prepared by nitrous acid depolymerization) that can accelerate thrombin inhibition by antithrombin III. The anti-thrombin activities of the octadecasaccharide and larger oligosaccharides were more readily neutralized by platelet factor 4 than were their anti-Factor Xa activities. These findings are compatible with two alternative mechanisms for the action of platelet factor 4, both involving the binding of the protein molecule adjacent to the antithrombin III-binding site. Such binding results in either steric interference with the formation of antithrombin III–proteinase complexes or in displacement of the antithrombin III molecule from the heparin chain.

The blood anticoagulant action of heparin is due to complex interactions between this polysaccharide and various proteins, i.e. the proteinase inhibitor antithrombin III and proteinases involved in the coagulation (clotting) mechanism (Rosenberg & Damus, 1973; Damus *et al.*, 1973; Björk & Lindahl, 1982). In whole blood this process may be modulated by the platelet-derived heparin-neutralizing protein platelet factor 4, which binds to heparin with high affinity (Handin & Cohen, 1976; Luscombe *et al.*, 1981; Jordan *et al.*, 1982). The structural requirements and functional implications of these various interactions are as yet ill-defined in several regards, but they have become increasingly amenable to experimental test, mainly owing to the development of methods for preparing heparin oligosaccharides of strictly defined molecular size.

Nomenclature used. No standardized nomenclature has been agreed to describe thrombin-inhibitory proteins or activities. Thus the major plasma proteinase inhibitor of thrombin is variously described as antithrombin or antithrombin III. Throughout the present paper the latter name is used. The heparin- or oligosaccharide-induced acceleration of thrombin inhibition by antithrombin III is termed (specific) anti-thrombin activity. Similarly, (specific) anti-Factor Xa activity is used to describe heparin- or oligosaccharide-induced acceleration of Factor Xa inhibition by antithrombin III.

Heparin potentiates the inhibitory action of antithrombin III against most of the proteinases of the blood coagulation and fibrinolytic systems (Damus *et al.*, 1973; Rosenberg, 1977). Only a fraction, about one-third, of the molecules in commercially available heparin preparations binds with high affinity to antithrombin III and accelerates the rates at which inhibitor–proteinase complexes are formed (Lam *et al.*, 1976; Höök *et al.*, 1976; Andersson *et al.*, 1976). The size and structure of the antithrombin III-binding region in these high-affinity heparin molecules have been the subject of a number of studies in recent years, all based on the use of nitrous acid to achieve controlled depolymerization of the polysaccharide (Rosenberg & Lam, 1979; Lindahl *et al.*, 1979, 1980; Oosta *et al.*, 1981; Riesenfeld *et al.*, 1981). After partial cleavage of heparin with this reagent, the smallest resulting fragment capable of binding with high affinity to antithrombin III was identified as an octasaccharide (Thunberg *et al.*, 1980), in which a unique pentasaccharide sequence constituted the actual binding region (Lindahl *et al.*, 1980, 1983; Casu *et al.*, 1981; Thunberg *et al.*, 1982; Björk & Lindahl, 1982).

Although the binding of heparin to antithrombin III is a prerequisite for the expression of anticoagulant activity, a number of studies have suggested that interaction between the polysaccharide and the target proteinase may also be of importance (Machovich *et al.*, 1975; Smith & Craft, 1976; Laurent *et al.*, 1978; Pomerantz & Owen, 1978; Holmer *et al.*, 1979; Thunberg *et al.*, 1979). Such a requirement was postulated for a group of coagulation proteinases, which included thrombin, on the basis of a positive correlation between inhibitory activity and molecular size of the polysaccharide (Jordan *et al.*, 1980; Holmer *et al.*, 1981). Other enzymes, represented by Factor Xa, did not conform to this relationship and were therefore considered not to interact with the heparin chain itself during complex-formation with antithrombin III. This difference was epitomized in inhibition studies with heparin oligosaccharides (with high affinity for antithrombin III) in the extreme small-M_r region, i.e. octa- to dodeca-saccharides (Holmer *et al.*, 1980). These compounds were thus essentially unable to potentiate the inhibition of thrombin, but showed anti-Factor Xa activities similar to that of intact high-affinity heparin. With increasing molecular size the oligosaccharides (obtained by deamination of heparin) were found to gain anti-thrombin activity; the smallest oligosaccharide with significant anti-thrombin activity was claimed to be a tetradecasaccharide (Oosta *et al.*, 1981) or a hexadecasaccharide (Stone *et al.*, 1982). These studies suggested, therefore, that the antithrombin III-binding region is essentially sufficient to potentiate the interaction of antithrombin III and Factor Xa, whereas structures in addition to this region are required for the interaction of heparin with thrombin.

The structural requirements for the interaction of heparin with platelet factor 4 are poorly defined. In a preliminary report Denton *et al.* (1983) showed that heparin oligosaccharides with high affinity for antithrombin III and small molecular size (predominantly 8–12 monosaccharide residues) were less amenable to neutralization by platelet factor 4 (as shown by the retention of anti-Factor Xa activity) than were the corresponding oligosaccharides of larger molecular size ($\geqslant$16 monosaccharide residues). In the present study we have examined the effects of platelet factor 4 on the interactions between heparin, antithrombin III and the target proteinases, Factor Xa or thrombin, with a series of well-defined oligosaccharides ranging from octasaccharide to octadecasaccharide in size.

Materials and methods

Oligosaccharides with high affinity for antithrombin III were isolated after partial deaminative cleavage of heparin with nitrous acid (30 min reaction period at −10°C), as described previously (Thunberg *et al.*, 1982). Products that remained bound to antithrombin III–Sepharose in 0.9 M-NaCl were recovered and separated by repeated gel chromatography on Sephadex G-50 (3 cm × 250 cm column) in 1 M-NaCl. The Sephadex G-50 column was calibrated with reference oligosaccharides (Thunberg *et al.*, 1980) in order that the oligosaccharide chain size could be determined. For further information see the legend to Fig. 1.

The concentration of heparin oligosaccharides was determined by the carbazole reaction for hexuronic acid (Bitter & Muir, 1962).

The effects of heparin oligosaccharides on the inhibition of thrombin or Factor Xa by antithrombin III were determined as described by Nordling & Björk (1980), with the use of the synthetic chromogenic peptide substrates S-2238 and S-2222 (KabiVitrum AB, Stockholm, Sweden) respectively to assay residual enzyme activity. Each oligosaccharide was tested at three or more different concentrations, generally below 1 μg/ml, and the results were related to the anti-thrombin and anti-Factor Xa activities of the 3rd International Heparin Standard. Similar assays were also performed with the use of human citrated plasma as a source of antithrombin III (Denton *et al.*, 1983).

The neutralization by platelet factor 4 (a gift from Dr. D. Pepper, S.E. Scotland Blood Transfusion Service, Edinburgh, Scotland, U.K.) of the

anti-thrombin and anti-Factor Xa activities of heparin oligosaccharides was studied as described previously (Denton *et al.*, 1983). Briefly, increasing amounts of platelet factor 4 (0–100 μg/0.1ml of 0.15M-NaCl/0.05M-Tris/HCl buffer, pH8.4) were added to tubes containing 1.0 μg of heparin or oligosaccharides in 0.9ml of the same buffer. The samples were preincubated for at least 2min and then processed to determine residual anti-Factor Xa or anti-thrombin activities as described above. A solution of heparin (1 μg/ml) without added platelet factor 4 was used as 0%-neutralization standard, and a solution of platelet factor 4 (10 μg/ml) containing no heparin was used to indicate 100% neutralization.

Results

Anti-thrombin and anti-Factor Xa activities of heparin oligosaccharides

A series of heparin oligosaccharides with high affinity for antithrombin III was isolated after partial deaminative cleavage of the polysaccharide with HNO_2. In order to ensure the occurrence in all molecules isolated of an intact antithrombin III-binding region, only components that remained bound to the immobilized proteinase inhibitor at 0.9M-NaCl concentration were retained for investigation (Thunberg *et al.*, 1982). The high-affinity preparation thus obtained was fractionated further by gel chromatography on Sephadex G-50, yielding distinct, albeit incompletely separated, peaks of octa-, deca-, dodeca-, tetradeca-, hexadeca- and octadeca-saccharides, in addition to a fraction of larger, poorly resolved, molecules (results not shown). The middle portion of each peak, as well as a fraction judged to contain molecules composed of >18 monosaccharide units, were recovered and rechromatographed separately on the same column (Fig. 1). The resulting chromatograms indicated essentially homogeneous fractions of up to octadecasaccharide size. The final yields of the various oligosaccharides were in the range 1–2mg for each fraction/g of heparin starting material, except for the octasaccharide (approx. 0.6mg/g). Reduction with NaB^3H_4 (Thunberg *et al.*, 1982) afforded labelled components that emerged well retarded in relation to intact high-affinity heparin on affinity chromatography (antithrombin III–Sepharose; results not shown). Furthermore, structural analysis of the octasaccharide, as reported previously (Lindahl *et al.*, 1980; Thunberg *et al.*, 1982), revealed the 3-*O*-sulphated glucosamine residue implicated as a unique component of the antithrombin III-binding region.

The effects of the isolated oligosaccharides on the inhibition of thrombin or Factor Xa by anti-

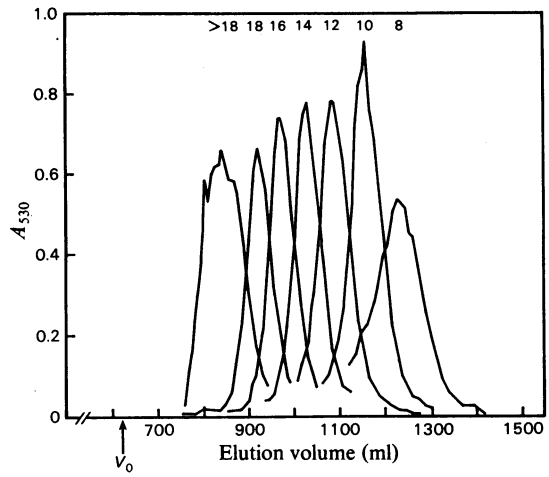

Fig. 1. *Gel chromatography of heparin oligosaccharides with high affinity for antithrombin III*

Heparin was depolymerized with nitrous acid and the products were fractionated by affinity chromatography on antithrombin III–Sepharose as described in the Materials and methods section. High-affinity components (approx. 30mg/g of starting material) were fractionated by gel chromatography on a column (3cm × 250cm) of Sephadex G-50 (superfine grade) equilibrated with 1M-NaCl. Effluent fractions were analysed for uronic acid by the carbazole reaction. Fractions corresponding to oligosaccharide peaks were pooled and re-applied separately to the same column; the Figure shows the resulting superimposed chromatograms. The fractions corresponding to the middle two-thirds of each peak (less of the octasaccharide) were recovered and desalted by passage through Sephadex G-15. The number of monosaccharide units per molecule is shown above each of the various oligosaccharide peaks. V_0, Void volume.

thrombin III were determined with the use of specific chromogenic substrates for these enzymes. The results obtained with assays based on purified antithrombin III (Fig. 2*a*), or with plasma substituted for the isolated inhibitor (Fig. 2*b*), were similar, except for the generally higher anti-Factor Xa activities recorded in the plasma system. This difference is probably due to the occurrence in plasma of lipoproteins that have been shown to neutralize preferentially the anti-Factor Xa activity of large-M_r heparins, such as that of the standard used in the assays (MacGregor *et al.*, 1979, 1980). The molar specific anti-Factor Xa activities determined in the purified assay system were fairly constant throughout the series of oligosaccharides and were in the range 1300–2200units/μmol (Fig. 2*a*). For comparison, the corresponding specific activity of undegraded high-affinity heparin (M_r approx. 13000) can be calculated as approx.

4000 units/μmol, suggesting that much of the anti-Factor Xa activity of the parent polysaccharide can be recovered in fragments containing little more than the antithrombin III-binding region. In contrast, with either assay system, no significant anti-thrombin activity was detectable for any of the oligosaccharides smaller than octadecasaccharide (Figs. 2*a* and 2*b*). The octadecasaccharide showed definite, albeit low, anti-thrombin activity, which was increased approx. 5-fold in the adjacent fraction containing somewhat larger oligosaccharides.

Neutralizaton of heparin oligosaccharides by platelet factor 4

The ability of purified platelet factor 4 to neutralize the anti-Factor Xa activities of the oligosaccharides was examined by determining residual activity after preincubation of oligosaccharides with the platelet protein at various concentrations. The resulting neutralization curves determined in the purified system or with plasma as a source of antithrombin III were essentially superimposable [normal citrated plasma contains small amounts of platelet factor 4, approx. 100 ng/ml (MacGregor *et al.*, 1979), but when appropriately diluted this is insufficient to influence the course of neutralization]. The neutralization of the oligosaccharides was compared with that of the 3rd International Heparin Standard (Fig. 3). Addition of increasing quantities of platelet factor 4 progressively neutralized the anti-Factor Xa activity of 1 μg of this standard, with 50% inhibition being achieved by approx. 1.4 μg of platelet factor 4. Total inhibition required approx. 6.6 μg of platelet factor 4. The amount of platelet factor 4 required for inhibition was 2-fold less than in the previous study (Denton *et al.*, 1983). This discrepancy, which could reflect either differences in inhibitory capacity of different preparations of platelet factor 4 or solubility problems with the protein, was not regarded as important, as the same batch of reconstituted platelet factor 4 was used throughout the present study.

Preincubation of platelet factor 4 with the heparin decasaccharide produced no detectable inhibition of the anti-Factor Xa activity at the protein concentrations tested (Fig. 3). In contrast, some neutralization of the tetradecasaccharide was observed with 5–100 μg of platelet factor 4; however, the maximal attainable degree of neutralization corresponded to less than 50% of the initial activity, even at high concentration of platelet factor 4. The hexadecasaccharide was more readily neutralized, 50% inhibition being achieved with 8 μg of platelet factor 4, but again showed some anti-Factor Xa activity that resisted neutralization (Fig. 3). The octadecasaccharide and larger oligosaccharides were completely neutralized at high concentrations of platelet factor 4, although the neutralization curves were displaced from that of the standard heparin, 5.8 μg of platelet factor 4 being required to achieve 50% neutralization.

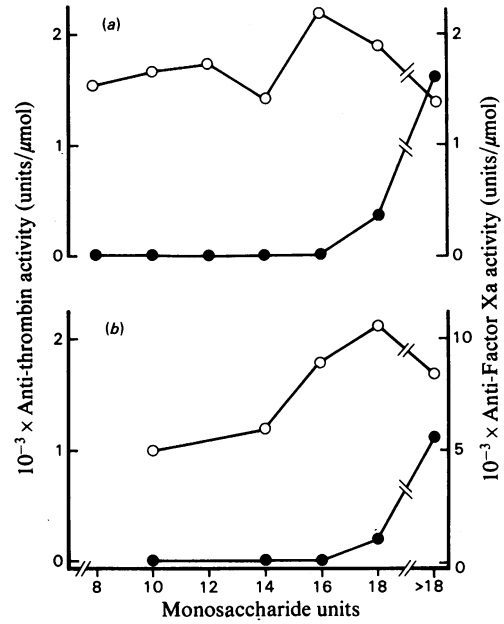

Fig. 2. *Anticoagulant activities of heparin oligosaccharides with high affinity for antithrombin III*

The isolated oligosaccharides were analysed for anti-thrombin (●) and for anti-Factor Xa (○) activity in a purified (*a*) or plasma (*b*) system. The results are expressed on a molar basis, assuming an M_r for a disaccharide of 600. No correction has been applied for the occurrence of under-sulphated residues in the antithrombin III-binding region. An M_r of 6000 has been assumed for the oligosaccharides indicated as containing >18 sugar units. For additional details see the Materials and methods section.

The effect of platelet factor 4 on the antithrombin activities of the octadecasaccharide and of the fraction of larger oligosaccharides was also studied (Fig. 4). Again, the 3rd International Heparin Standard was used for comparison. The anti-thrombin activity of the standard heparin was neutralized in a similar manner to its anti-Factor Xa activity (50% inhibition of 1 μg of the standard by 1.4 μg of platelet factor 4) but with a slightly steeper sigmoidal neutralization curve. The antithrombin activities of the octadecasaccharide and of the larger oligosaccharides could be completely abolished by the addition of platelet factor 4. The neutralization curves were essentially similar to that observed with the heparin standard (Fig. 4), 50% inhibition of 1 μg of each saccharide requiring 1.2 and 2.1 μg of platelet factor 4 respectively.

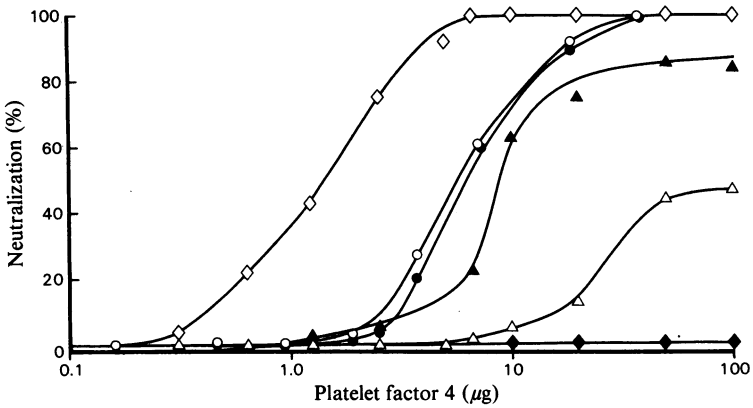

Fig. 3. *Neutralization of the anti-Factor Xa activities of the heparin oligosaccharides by increasing quantities of platelet factor 4*
To incubation mixtures of antithrombin III and Factor Xa were added the oligosaccharides (1 μg) or oligosaccharides preincubated with increasing quantities of platelet factor 4. Residual anti-Factor Xa activities of the oligosaccharides were then determined with the substrate S2222. ◊, 3rd International Heparin Standard; ♦, decasaccharide; △, tetradecasaccharide; ▲, hexadecasaccharide; ○, octadecasaccharide; ●, preparation of larger oligosaccharides.

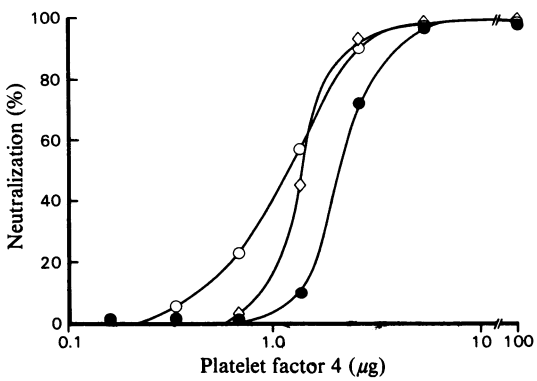

Fig. 4. *Neutralization of the anti-thrombin activities of the heparin oligosaccharides by increasing quantities of platelet factor 4*
To incubation mixtures of antithrombin III and thrombin were added the oligosaccharides (1 μg) or oligosaccharides preincubated with increasing quantities of platelet factor 4. Residual anti-thrombin activities of the oligosaccharides were then determined with the substrate S2238. ◊, 3rd International Heparin Standard; ○, octadecasaccharide; ●, preparation of larger oligosaccharides.

Discussion

The smallest oligosaccharide capable of potentiating the inhibition of thrombin has been claimed to be a tetradecasaccharide (Oosta *et al.*, 1981) or a hexadecasaccharide (Stone *et al.*, 1982). However, these reports did not describe any attempts to ascertain the size homogeneity of the alleged tetradeca- or hexadeca-saccharide fractions, which could therefore have been contaminated by larger molecules. The preparation procedure employed in the present study provided essentially monodisperse oligosaccharide fractions of up to octadecasaccharide size, as verified by separate gel chromatography of each isolated fraction (Fig. 1). The invariably high anti-Factor Xa activities of these oligosaccharides (Fig. 2) conform to the notion (Holmer *et al.*, 1980; Oosta *et al.*, 1981; Casu *et al.*, 1981) that the saccharide structure required to potentiate the inactivation of Factor Xa is essentially contained within the antithrombin III-binding region of the heparin molecule. However, significant anti-thrombin activity was found to be associated only with the octadecasaccharide and the larger oligosaccharides, but not with the smaller species such as tetradeca- or hexadeca-saccharides (Fig. 2). These findings strongly suggest that an octadecasaccharide is the smallest deamination product of heparin that will span the saccharide sequence required to potentiate the inactivation of thrombin by antithrombin III. Although part of this sequence is presumably occupied by the antithrombin III-binding pentasaccharide region, the structure (and function) of the remaining portion is unclear. A number of investigators have proposed that direct binding of thrombin to the heparin molecule, adjacent to antithrombin III, promotes the inhibition of the proteinase (Laurent *et al.*, 1978; Machovich & Aranyi, 1978; Pomerantz & Owen, 1978; Holmer *et al.*, 1979;

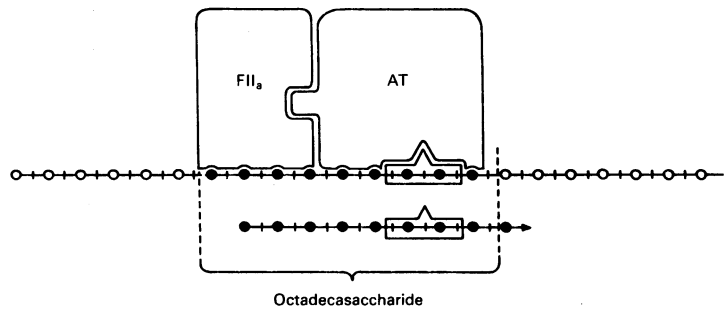

Fig. 5. *Proposed model of the interaction between heparin, antithrombin III (AT) and thrombin (FIIa)*
The model illustrates the formation of a ternary complex involving an octadecasaccharide sequence of the heparin chain (within the bracket, ● denoting uronic acid residues). The specific antithrombin III-binding pentasaccharide sequence is indicated, as well as the interaction between thrombin and heparin. The size and structural characteristics of the thrombin-binding region in the heparin molecule are unknown. Displacement of the antithrombin III-binding sequence within the octadecasaccharide results in an incomplete binding site for thrombin. One example of such a functionally inadequate oligosaccharide is indicated beneath the ternary complex. The arrowhead on this latter oligosaccharide denotes a 2,5-anhydromannose residue formed during nitrous acid depolymerization; the thrombin-binding region is arbitrarily assumed to be located towards the non-reducing end of the heparin chain, relative to the antithrombin III-binding sequence.

Oosta *et al.*, 1981). Moreover, Oosta *et al.* (1981) studied the kinetic properties of the aforementioned tetradeca- and hexadeca-saccharide fractions in reactions with antithrombin III and thrombin, and postulated a second site of interaction between heparin and antithrombin III, committed to activating specifically the inhibitor against thrombin. Pending further corroboration of this proposal, it seems reasonable at present to limit the discussion to two protein-binding regions in the heparin molecule, one, presumably a pentasaccharide sequence, that binds (and activates) antithrombin III, and another that binds thrombin (Fig. 5). The size of the latter region is unknown; however, our results indicate that both regions can be accommodated in an octadecasaccharide, but not in a hexadecasaccharide, deamination fragment of heparin. The relatively low anti-thrombin activity of the octadecasaccharide, as compared with that of the larger oligosaccharides (Fig. 2), may be explained in terms of the random nature of the deaminative cleavage reaction used to generate the oligosaccharides. Since hexadecasaccharides are inactive, it is obvious that only those octadecasaccharides in which the antithrombin III-binding region is maximally shifted towards one end of the molecule can also contain a functional thrombin-binding region. This is illustrated in Fig. 5, which shows an octadecasaccharide that lies, by one disaccharide unit, outside the appropriate frame and will therefore be unable to potentiate the inactivation of thrombin. Clearly, of the various potential octadecasaccharides with high affinity for antithrombin III only a minor fraction will also contain the thrombin-binding region.

The differential effects of heparin oligosaccharides on the inhibition of coagulation enzymes may help to explain the mode of action of platelet factor 4. This protein forms complexes with heparin, the composition of which varies with the length of the polysaccharide molecule (Bock *et al.*, 1980). However, the mechanism by which platelet factor 4 inhibits the anti-Factor Xa and anti-thrombin activities of heparin has remained unclear. Specific interaction of platelet factor 4 with the antithrombin III-binding region of the heparin molecule appears unlikely, since the protein binds with apparently equal strength to heparin with high or with low affinity for antithrombin III (Niewiarowski *et al.*, 1979). Moreover, the results obtained in the present study, and in a previous investigation (Denton *et al.*, 1983), demonstrate that platelet factor 4 is unable to neutralize the anti-Factor Xa activity of an oligosaccharide approximately the size of the antithrombin III-binding region. The possible interactions of such an oligosaccharide are thus restricted to binding of antithrombin III, which is thereby activated to inhibit Factor Xa (Fig. 6*a*). This oligosaccharide is too small to bind either thrombin or platelet factor 4 alongside the antithrombin III molecule (Fig. 6*b*); it is therefore unable to potentiate the inactivation of thrombin and is resistant to neutralization by platelet factor 4. Conversely, a full-sized heparin chain potentiates the inactivation of both proteinases (Fig. 6*c*), since the additional saccharide sequence adjacent to the antithrombin III-binding region is required for thrombin-binding. The effects of platelet factor 4 on these interactions may be due to two different, though not necessarily

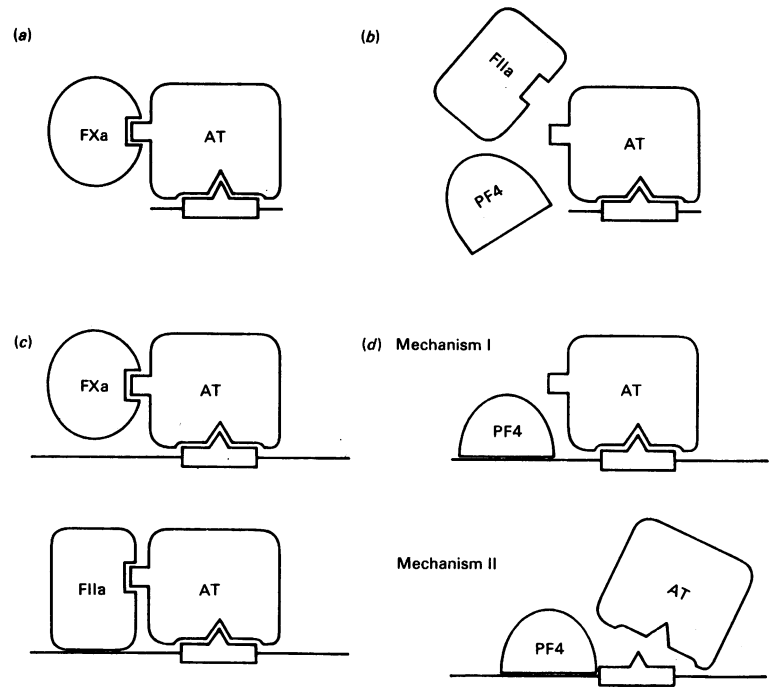

Fig. 6. *Possible interactions of an oligosaccharide, the approximate size of the antithrombin III-binding region, and of a full-sized heparin chain, with antithrombin III, Factor Xa, thrombin and platelet factor 4*

An oligosaccharide containing essentially only the antithrombin III-binding sequence binds to antithrombin III (AT) and accelerates the inhibition of Factor Xa (FXa) (*a*) but not thrombin (FIIa) (*b*). It cannot bind to platelet factor 4 (PF4) (*b*), and its anti-Factor Xa activity is therefore resistant to neutralization. A full-sized heparin chain can accommodate both antithrombin III and thrombin and therefore accelerates the inhibition of both Factor Xa and thrombin (*c*). Platelet factor 4 binds to full-sized heparin and neutralizes its anti-Factor Xa and anti-thrombin activities (*d*). Two mechanisms can be proposed to explain its neutralizing action. In Mechanism I platelet factor 4 binds to a site adjacent to the antithrombin III-binding site and sterically hinders formation of the antithrombin III–proteinase complex. Alternatively, in Mechanism II platelet factor 4 binds immediately adjacent to the antithrombin III-binding site and displaces the inhibitor from the polysaccharide chain. Binding of platelet factor 4 to a heparin chain by Mechanism II may possibly take place on either side of the antithrombin III-binding site (provided that the size of the chain and location of the antithrombin III-binding site are suitable), although this is not depicted in the diagram.

mutually exclusive, mechanisms (Fig. 6*d*). The protein may bind to the polysaccharide chain in such a way as to cause a steric hindrance to the appropriate interaction of the proteinases, thrombin or Factor Xa, with the heparin–antithrombin III complex (Mechanism I). Alternatively, binding of platelet factor 4 to heparin directly alongside the antithrombin III-binding region (or at a site that partially or completely includes this region) may lead to displacement of the proteinase inhibitor from the polysaccharide chain (Mechanism II). The information available does not permit any conclusive discrimination between these alternatives. One may note that the anti-Factor Xa activity of the tetradeca- and hexadeca-saccharide fractions was only partially neutralized by platelet factor 4. This observation probably reflects the variable location of the antithrombin III-binding region in the molecules. The location of this region will determine the sizes of the adjacent, residual, saccharide sequences on either side, and thus the conditions for binding of platelet factor 4 molecules. This structural and functional heterogeneity of oligosaccharides that are homogeneous with regard to molecular size further supports the conclusion that platelet factor 4 may bind to heparin adjacent to the antithrombin III-binding region and thereby modulate the various anticoagulant activities of the polysaccharide.

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