Effect of oversulphated chondroitin and dermatan sulphate upon thrombin and Factor Xa inactivation by antithrombin III or heparin cofactor II

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The kinetics of inhibition of human thrombin and Factor Xa by antithrombin III or heparin cofactor II were examined under pseudo-first-order conditions as a function of the concentration of naturally occurring oversulphated chondroitin and dermatan sulphates. The sulphated glycosaminoglycans (GAGs) studied were chondroitin sulphate D (CSD) (GlcA-2-SO₄-GalNAc-6-SO₄), chondroitin sulphate K (CSK) (GlcA-3-SO₄-GalNAc-4-SO₄), chondroitin sulphate H (CSH) (IdA-GalNAc-4,6-diSO₄) and polysulphated dermatan sulphate (DPS) (IdA-2-SO₄ or -3-SO₄-GalNAc-4,6-diSO₄). The data for the antithrombin III inhibition of thrombin showed a low degree of maximal potentiation of this interaction (≈ 10 -fold), which would appear to be characteristic of GAGs devoid of the high-affinity antithrombin III binding site. In contrast there was a greater potentiation of the inhibition of thrombin by heparin cofactor II with DPS showing an activity comparable to heparin in this interaction at a concentration two orders of magnitude lower than dermatan sulphate. DPS potentiated antithrombin III–Factor Xa interaction by 1200-fold, similar to that shown by high-affinity heparin of 6 kDa. The antithrombin III–Factor Xa interaction was potentiated by all other GAGs studied to a degree similar to that of heparin pentasaccharide with high affinity for antithrombin III. The findings suggest more stringent structural requirements for GAG stimulation of antithrombin–thrombin interaction than for antithrombin–Factor Xa or heparin cofactor–thrombin interaction, which may also be of significance in physiological control of haemostasis.

INTRODUCTION

A number of reports have shown that deficiency of the protease inhibitors, antithrombin III and heparin cofactor II, is associated with thrombotic diseases [1–4]. The rate of interaction between these inhibitors (at plasma concentrations) and their target enzymes, e.g. thrombin or coagulation Factor Xa, has been shown to be rather slow and well outside the minimum proposed to exert physiological control [5]. To achieve this minimum rate it is necessary to envisage acceleration, presumably by heparin or heparin-like compounds, in or about the vasculature [6]. In this context, heparin shows unique properties: accelerating antithrombin III–thrombin interaction 10000-fold [7]; antithrombin III–Factor Xa interaction 4500-fold [8], and heparin cofactor II–thrombin interaction 800-fold [9], at concentrations between 10⁻⁷ and 10⁻⁸ M. However, heparin is localized in mast cells and would not appear to be readily available for control of haemostasis [10]. Other candidates for a role in this control are the sulphated glycosaminoglycans (GAGs) lining and within the vessel wall (chondroitin and dermatan sulphate comprising 80–90% of the total GAG content) and heparan sulphates [6,11,12]. Chondroitins 4- and 6-sulphate have little or no anticoagulant properties [13] and dermatan sulphate, although causing 500-fold acceleration of heparin cofactor II–thrombin

interaction, does so only at very high concentrations [9]. Charge-density properties related primarily to the sulphate content of GAGs appear to play a significant part in the anticoagulant potency of heparin and heparinoids [14,15], and in this present paper we have investigated the ability of naturally occurring oversulphated forms of chondroitin sulphate and dermatan sulphate to accelerate the interaction between these protease inhibitors and the target coagulation proteases.

MATERIALS AND METHODS

Human thrombin, Factor Xa, antithrombin III and heparin cofactor II were prepared as described previously [16–18]. Chondroitin sulphate D (CSD) (GlcA-2-SO₄-GalNAc-6-SO₄) was purified from shark cartilage [19]; chondroitin sulphate K (CSK) (GlcA-3-SO₄-GalNAc-4-SO₄) from king-crab cartilage [19]; chondroitin sulphate H (CSH) (IdA-GalNAc-4,6-diSO₄) from hagfish notochord [19,20] and trisulphated (polysulphated) dermatan sulphate (DPS) (IdA-2-SO₄ or -3-SO₄-GalNAc-4,6-diSO₄) from hagfish skin [21]. The sulphate/hexosamine ratio for each of the GAGs was as follows: CSD, 1.52; CSK, 1.54; CSH, 1.89; DPS, 2.1. The molecules consist, therefore, of mixtures of disaccharide units which may be unsulphated and mono-, di- or tri-sulphated according to overall sulphate content [22].

Abbreviations used: GAGs, glycosaminoglycans; CSD, CSK, CSH and CSE, chondroitins sulphate D, K, H and E, respectively; DPS, polysulphated dermatan sulphate.

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Kinetics of interaction between protease inhibitors and coagulation proteases

The rate of interaction between antithrombin III or heparin cofactor II and thrombin in the presence of increasing concentrations of each GAG was measured using the thrombin chromogenic substrate, S2238 (Flow Laboratories, Rickmansworth, U.K.) [23,24]. The kinetics of interaction of antithrombin III–Factor Xa were also measured using a chromogenic substrate, S2222 (Flow Laboratories) [8]. A mixture of inhibitor and substrate in buffer (for details see the legends to Figs. 1, 2 and 3) was prewarmed to 37 °C in plastic optical cuvettes in an Abbott Bichromatic Analyser (with 380/450 nm filter) before addition of enzyme at time zero. Under the conditions employed (protease inhibitor $\gg$ enzyme) the rate of reaction follows first-order kinetics such that:

$$k^1 = k''_{(\text{protease inhibitor})}$$

k'' being the second-order rate constant for interaction between the inhibitor and enzyme. The reaction is followed by inclusion of substrate, so there is competitive inhibition of the interaction according to the concentration of the substrate and its K_m with the enzyme such that:

$$k'_{\text{obs.}} = \frac{k''_{(\text{protease inhibitor})}}{1 + \frac{[S]}{K_m}}$$

First-order rate constants were calculated from semi-logarithmic plots of V_1 against time where:

$$V_1 = \frac{V}{V_0}$$

where V is the rate of substrate hydrolysis at time t and V_0 is the rate of hydrolysis at time zero (measured in the absence of inhibitor). The maximum rate of interaction between inhibitor and enzyme, and the concentration of GAG at which half the maximal rate is achieved were estimated by plotting the inverse of the rate against the inverse of the GAG concentration as described previously [23].

RESULTS

Antithrombin III–thrombin interaction

Fig. 1 shows the progressive inhibition of thrombin by antithrombin III in the presence of various sulphated GAGs (100 $\mu\text{g}/\text{ml}$). The rate of interaction in the absence of GAG was estimated as corresponding to a second-order rate constant of $3.5 \times 10^5 \text{ M}^{-1} \cdot \text{min}^{-1}$ [25]. CSD showed no activity under these conditions. The acceleration of rate observed in the presence of CSK, CSH and DPS was $2.24 \times 10^6 \text{ M}^{-1} \cdot \text{min}^{-1}$, $2.8 \times 10^6 \text{ M}^{-1} \cdot \text{min}^{-1}$ and $5.6 \times 10^6 \text{ M}^{-1} \cdot \text{min}^{-1}$, that is 6.4-fold, 8-fold and 16-fold over basal rate, respectively. Upon reduction of the concentration of DPS, there was a very gradual decline in the observed rate and the concentration at which it was half-maximal was determined as 3 $\mu\text{g}/\text{ml}$ or 0.15 μM . This can be compared with values of 4 nM for heparin [26] (average molecular mass and high affinity for antithrombin III) and 3.6 μM for pentosan polysulphate [23]. An even shallower decrease in rate was observed with reduction in the concentrations of CSK and CSH, and half-maximal decrease in rate had not occurred at 8 $\mu\text{g}/\text{ml}$ and 1 $\mu\text{g}/\text{ml}$, respectively.

Fig. 1. Effect of various GAGs on the interaction between antithrombin III and human α -thrombin

Thrombin and antithrombin III at final concentrations of 10 nM and 10 μM , respectively were mixed in the presence of substrate S2238 (final concentration 0.16 mM) and 100 $\mu\text{g}/\text{ml}$ of GAG. □, CSK; ▲, CSH; ○, DPS; ●, no addition. Buffer was 0.1 M-triethanolamine/HCl (pH 7.8)/0.1 M-NaCl containing 0.1% (w/v) polyethylene glycol 6000. The rate of *p*-nitroaniline release, expressed as V_1 (see Materials and methods section) is plotted against the time of incubation.

Antithrombin III–Factor Xa interaction

The inhibition of Factor Xa by antithrombin III was potentiated by all the GAGs (Fig. 2). The second-order rate constant in the absence of GAG and presence of calcium (5 mM) was $6.3 \times 10^4 \text{ M}^{-1} \cdot \text{min}^{-1}$ as we have shown previously [27]. The maximal second-order rate constant in the presence of GAG was: DPS, $7.6 \times 10^7 \text{ M}^{-1} \cdot \text{min}^{-1}$; CSH, $5.7 \times 10^6 \text{ M}^{-1} \cdot \text{min}^{-1}$; CSK, $1.8 \times 10^7 \text{ M}^{-1} \cdot \text{min}^{-1}$ and CSD, $1.1 \times 10^7 \text{ M}^{-1} \cdot \text{min}^{-1}$. DPS showed a maximal acceleration of 1200-fold and half this rate was achieved at a concentration of 0.5 $\mu\text{g}/\text{ml}$ (25 nM). This can be compared with a maximal 360-fold acceleration by dermatan sulphate at 200 $\mu\text{g}/\text{ml}$ (data not shown) or 190-fold acceleration at 1.0 $\mu\text{g}/\text{ml}$ [8] for a pentasaccharide with high affinity for antithrombin III [28]. The properties of DPS are, in fact, more comparable to a high-affinity heparin of molecular mass 6 kDa [8]. Maximum acceleration by CSH was 90-fold over basal rate, similar to the maximum 100–200-fold acceleration of the antithrombin III–Factor Xa interaction which we have observed for chondroitin sulphate E (CSE) (GlcA-Gal-NAc-4,6-diSO₄) [29], pentosan polysulphate, a heparan sulphate, heparin pentasaccharide (Table 1) and also have found herein for CSK and CSD. The 13-fold

Fig. 2. Effect of various concentrations of GAGs upon the interaction between antithrombin III and Factor Xa

The second-order rate constants for the inhibition of Factor Xa are plotted against concentrations of GAG on a double logarithmic scale. The second-order rate constant was calculated from the rate of inhibition of 3.6 nM-Factor Xa by 30 nM-antithrombin III in 0.05 M-Tris/HCl (pH 7.4)/0.1 M-NaCl/5 mM-CaCl₂/0.1 % (w/v) polyethylene glycol 6000 at 37 °C, in the presence of chromogenic substrate S2337 at a concentration of 0.5 mM [8]. ▼, DPS; △, CSH; □, CSK; ◆, CSD; ●, unfractionated heparin with high affinity for antithrombin III; ■, heparin pentasaccharide with high affinity for antithrombin III.

Table 1. Maximal-fold acceleration of rate by various sulphated polysaccharides calculated by reference to the rate of interaction between inhibitor (antithrombin III and heparin cofactor II) and coagulation protease in their absence as described previously [23]

Numbers in brackets refer to references from which the data is taken. ATIII, antithrombin III; HCII, heparin cofactor II; Xa, Factor Xa; Th, thrombin; NT, not listed.

	Acceleration of inhibition (- fold)		
	ATIII-Th	ATIII-Xa	HCII-Th
Heparin, unfractionated*	3220[26]	4500[8]	840[26]
Heparin, 22 kDa	5775[26]	6666[8]	1800[26]
Heparin, 11 kDa	3770[26]	4700[8]	1050[26]
Heparin, pentasaccharide	0[26]	190[8]	0[26]
DPS	16	1200	840
CSH	8	90	76
Dermatan sulphate (porcine skin)	NT	360	420[31]
Dermatan sulphate (beef lung)	NT	NT	280[31]
CSK	6.4	280	116
CSD	0	180	3
CSE	11[29]	150[29]	185[29]
Pentosan polysulphate	40[23]	200[23]	500[24]
Endoglycan†	11[29]	180[29]	500[29]

* Heparin preparations are of high affinity for antithrombin III.

† Endoglycan is a pharmaceutical preparation (Mediolanum Pharmaceuticals, Milan, Italy) containing equal proportions of heparan sulphate and dermatan sulphate as isolated from bovine aortic intima.

Fig. 3. Effect of GAGs on the rate constant for the inhibition of α-thrombin by heparin cofactor II

The second-order rate constant for the inhibition of α-thrombin is plotted against the concentration of GAG on a single logarithmic scale (demonstrating the bell-shaped nature of activity against concentration). The second-order rate constant was calculated from the rate of inhibition of 7.5 nM-thrombin by 37.5 mM-heparin cofactor II in 0.1 M-triethanolamine/HCl (pH 7.8)/0.1 M-NaCl containing 0.1 % (w/v) polyethylene glycol 6000, in the presence of chromogenic substrate S2238 (final concentration 0.133 mM). □, DPS; ●, unfractionated heparin; ▲, dermatan sulphate (porcine skin).

higher acceleration by DPS over that shown by CSH may be due to the higher degree of sulphation of DPS (SO₄/hexosamine ratio: CSH, 1.84; DPS, 2.1) or the presence in the molecule of IdA-2-SO₄ or IdA-3-SO₄; structures which have been shown to be important in binding of Factor Xa to heparin [30].

Heparin cofactor II–thrombin interaction

The second-order rate constant for this reaction was $5 \times 10^5 \text{ M}^{-1} \cdot \text{min}^{-1}$ in the absence of GAG [9]. DPS accelerated the reaction to a maximum which corresponded to a second-order rate constant of $4.2 \times 10^8 \text{ M}^{-1} \cdot \text{min}^{-1}$, similar to unfractionated heparin (Fig. 3) [26,31]. Dermatan sulphate achieved a high rate ($2.15 \times 10^8 \text{ M}^{-1} \cdot \text{min}^{-1}$), but at a 100-fold higher concentration than DPS [9,31]. The bell-shaped curve obtained by plotting activity against concentration of DPS is similar to that shown by heparin and is indicative of a template mechanism for acceleration of the reaction [32]. The mechanism is dependent on binding to both inhibitor and protease; at saturating levels of GAG, inhibitor and protease are bound to separate molecules which is observed as a decrease in reaction rate.

The acceleration by CSD, CSK and CSH is somewhat weaker than has been observed for other highly sulphated polysaccharides [17,18,31] (Table 1) and suggests a degree of specificity for such a property. Thus, the sulphation of the 6-OH of GalNAc (CSH) reduces the activity relative to dermatan sulphate, but further sulphation on the 2- or 3-OH of iduronate results in a highly potent activator of heparin cofactor II–thrombin interaction.

DISCUSSION

The interaction between antithrombin III and thrombin is known to occur in two steps: first, the formation of a Michaelis-type complex between the two proteins and then a slower transformation in which the thrombin is irreversibly bound. The mechanism by which heparin causes acceleration of the rate of this reaction has been shown to be due to a 1000-fold increase in the stability of the Michaelis complex with no effect on the second stage [33]. A conformational change induced on specific binding of heparin by the inhibitor enhances its receptivity to binding to the enzyme [34,35]. There is also a template effect by which the ionic binding of protease enhances its interaction with that fraction of inhibitor bound to the same molecule [36–38]. A key structural feature of the binding to antithrombin III is a specific pentasaccharide sequence on the heparin which tightly binds to the inhibitor and induces the conformational change [28,39]. Other structural features of the heparin molecule of importance are the molecular mass, which influences heparin action as a template [40], and also charge density, which would appear to be involved in binding of the protease [14,15,41]. The minimal molecular mass for heparin to accelerate the antithrombin III–thrombin interaction is 5–6 kDa and no activity is observed below this molecular mass [40]. Acceleration of the antithrombin III–Factor Xa interaction occurs when a heparin pentasaccharide with high affinity for antithrombin III binds to the inhibitor [28]. For this reason it has been concluded that conformational change alone is sufficient to cause acceleration of this reaction. However, our own observations show that highest acceleration occurs only with heparin of molecular mass greater than 11 kDa [8]. This enhancement of activity is lost in the presence of higher ionic strength (> 0.15 M-NaCl) or when Factor Xa is bound in the prothrombinase complex, suggesting possible involvement of Factor Xa binding (template effect) in the mechanism. Heparin can also cause acceleration of the rate of interaction between heparin cofactor II and thrombin. The mechanism would appear to be similar to antithrombin III in that a template effect has been demonstrated [26,32] and increase in charge density is associated with increased potency [41]. However, binding between inhibitor and heparin is susceptible to disruption by low concentrations of salt (> 0.15 M) [26] and evidence for a specific interactive site between inhibitor and GAG is not fully established [42,43]. Dermatan sulphate is an activator causing similar acceleration to heparin but at higher concentration [9,31]. Also a number of synthetic heparinoids are potent activators of heparin cofactor II [18,24].

In this present report we have examined the properties of a number of oversulphated chondroitin and dermatan sulphates previously noted to have anticoagulant properties *in vitro* [44], and which have charge densities (measured as $\text{SO}_4/\text{hexosamine}$ ratio) of greater than 1.0 and approaching that of heparin. They all have a molecular mass greater than that found previously to be necessary for template action of heparin (antithrombin III–thrombin approximately 5–6 kDa [37]; antithrombin III–Factor Xa 10 kDa [8]). Chemically these GAGs are of an heterogeneous structure having a percentage of oversulphated disaccharides along a parent chondroitin 4- or 6-sulphate or dermatan sulphate backbone.

From our observations, we suggest that to accelerate significantly the rate of interaction between antithrombin III and thrombin, it is necessary that the GAG structure be able to bind specifically to, and cause conformational change in, antithrombin III and also to act as template by binding to thrombin. This is demonstrated in Table 1 which shows that significant acceleration of antithrombin III–thrombin interaction is catalysed only by heparin of sufficient molecular mass. DPS has a $\text{SO}_4/\text{hexosamine}$ ratio very close to that of heparin and yet is ineffective in accelerating this reaction. Our observation does not lend support to the hypothesis that antithrombin III–thrombin acceleration can be explained entirely from physicochemical theory and that it is related solely to a minimal linear charge density [15]. Our conclusion is supported also by previous observations on the properties of pentosan polysulphate, which has a higher charge density than heparin [23,31]. None of the GAGs tested caused any change in the conformation of antithrombin III as measured by a change in intrinsic fluorescence upon binding (data not shown). The binding affinity of the GAGs for thrombin was also tested as we have described previously, by measuring interference in heparin-catalysed antithrombin III–thrombin interaction [17], and found for CSK, CSH and DPS to be in the range of 10^{-7} – 10^{-6} M (data not shown), as has been observed for heparin [45,46] and previously by us for pentosan polysulphate [17].

Although we observed these various GAGs to have little effect on the antithrombin III–thrombin reaction, they all caused very significant acceleration of antithrombin III–Factor Xa interaction at low concentrations. This acceleration was similar to that seen with the synthetic heparin pentasaccharide with high affinity for antithrombin III [8]. We suggest, therefore, from these present results that acceleration of antithrombin III–Factor Xa interaction (unlike that between the inhibitor and thrombin) does not have an absolute prerequisite for specific binding to the inhibitor. With these molecules (also shown with CSE and pentosan polysulphate, Table 1), acceleration comparable to heparin pentasaccharide can occur at low concentrations of GAG. Interestingly, the potency of DPS was found to be of an order of magnitude higher (comparable to 6 kDa heparin) than the other glycosaminoglycans tested, which may be attributable to the presence in its structure of IdA-2-O- SO_4 shown to be of importance in binding heparin to Factor Xa [30].

Our observations upon the influence of these GAGs in acceleration of heparin cofactor II–thrombin interaction may suggest that there is an element of specificity greater than previously supposed. A previous conclusion that there is less need for specific GAG structure arose from observations that synthetic highly sulphated heparinoids (e.g. pentosan polysulphate, dextran sulphate) of widely different structure were potent activators of heparin cofactor II [18,24,31]. The results reported here lead to the conclusion that possession of high charge density is not the only requirement and the spatial disposition of these and other groups are of importance.

In conclusion, we have demonstrated that the ability to accelerate antithrombin III–Factor Xa or heparin cofactor II–protease interaction, which for chondroitin 4- and 6-sulphate is negligible [13], is considerably increased in oversulphated naturally occurring forms. In acceleration of antithrombin III–Factor Xa interaction,

this potency approaches that of heparin pentasaccharide. Dermatan sulphate has weak properties, but these are increased to be comparable to heparin if oversulphation introduces IdA-2-O-SO₄ or -3-O-SO₄ into the structure. Chondroitin and dermatan sulphate occur in high concentrations in the vasculature and whether oversulphated forms of these occur in this situation (oversulphated chondroitin sulphate is found in mammalian mast cells [47]) and play some part in the control of haemostasis is at present under investigation.

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REFERENCES

- Egeberg, O. (1965) *Thromb. Diath. Haemorrh.* **13**, 516–530
- Marciniak, E., Farley, C. H. & Desimone, P. A. (1974) *Blood* **43**, 219–231
- Scully, M. F., De Haas, H., Chan, P. & Kakkar, V. V. (1981) *Br. J. Haematol.* **47**, 235–240
- Tran, T. H., Marbet, G. A. & Duckert, F. (1985) *Lancet* **ii**, 413–414
- Travis, J. & Salvesen, G. S. (1983) *Annu. Rev. Biochem.* **52**, 655–709
- Marcum, J. & Rosenberg, R. (1984) *Biochemistry* **23**, 1730–1737
- Hoylaerts, M., Owen, W. G. & Collen, D. (1984) *J. Biol. Chem.* **259**, 5670–5677
- Ellis, V., Scully, M. F. & Kakkar, V. V. (1986) *Biochem. J.* **238**, 329–332
- Tollefsen, D. M., Pestka, C. A. & Monafu, W. J. (1983) *J. Biol. Chem.* **258**, 6713–6716
- Scully, M. F., Ellis, V. & Kakkar, V. V. (1986) *Lancet* **ii**, 718–719
- Marcum, J. A., Atha, D. H., Fritze, L. M., Nawroth, P., Stern, D. & Rosenberg, R. D. (1986) *J. Biol. Chem.* **261**, 7507–7517
- Greismacher, A., Hennes, R., Keller, R. & Greiling, H. (1987) *Eur. J. Biochem.* **168**, 95–101
- Teien, A. N., Abildgaard, U. & Hook, M. (1976) *Thromb. Res.* **8**, 859–867
- Hurst, R. E., Menter, J. M., Settine, J. M., West, S. S. & Coyne, E. H. (1979) *Biochemistry* **18**, 4283–4287
- Heuck, C. C., Schiele, U., Horn, D., Fronda, D. & Ritz, E. (1985) *J. Biol. Chem.* **260**, 4598–4603
- Ellis, V., Scully, M. F., MacGregor, I. & Kakkar, V. V. (1982) *Biochim. Biophys. Acta* **701**, 24–31
- Scully, M. F. & Kakkar, V. V. (1984) *Biochem. J.* **218**, 657–665
- Yamagishi, R., Niwea, M., Kondo, S., Sakuragawa, N. & Koide, T. (1984) *Thromb. Res.* **36**, 633–642
- Seno, N. & Murakami, K. (1982) *Carbohydr. Res.* **103**, 190–194
- Anno, K., Seno, N., Mathews, M. B., Yamagata, T. & Susuki, S. (1971) *Biochim. Biophys. Acta* **237**, 173–177
- Abiyama, F. & Seno, N. (1981) *Biochim. Biophys. Acta* **674**, 289–296
- Seldin, D. C., Seno, N., Austen, K. F. & Stevens, R. L. (1984) *Anal. Biochem.* **141**, 291–300
- Scully, M. F. & Kakkar, V. V. (1984) *Biochem. J.* **222**, 571–578
- Scully, M. F. & Kakkar, V. V. (1984) *Thromb. Res.* **36**, 187–194
- Downing, M. R., Bloom, J. W. & Mann, K. G. (1978) *Biochemistry* **17**, 2649–2653
- Scully, M. F., Ellis, V. & Kakkar, V. V. (1987) *Thromb. Res.* **46**, 491–502
- Ellis, V., Scully, M. F. & Kakkar, V. V. (1983) *Biochim. Biophys. Acta* **747**, 123–129
- Choay, J., Petitou, M., Lormeau, J. C., Sinay, P., Casu, B. & Gatti, G. (1983) *Biochem. Biophys. Res. Commun.* **116**, 492–499
- Scully, M. F., Ellis, V., Seno, N. & Kakkar, V. V. (1986) *Biochem. Biophys. Res. Commun.* **137**, 15–22
- Fransson, L. A., Havsmark, B., Nieduszynski, I. A., Huckerby, T. N. & Lewis, W. E. (1982) *Carbohydr. Res.* **104**, 139–146
- Scully, M. F., Ellis, V. & Kakkar, V. V. (1985) *Thromb. Res.* **41**, 489–499
- Griffith, M. J. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5460–5464
- Olson, S. T. & Shore, J. D. (1982) *J. Biol. Chem.* **257**, 14891–14895
- Nordenman, B., Danielsson, A. & Bjork, I. (1978) *Eur. J. Biochem.* **90**, 1–6
- Einarsson, R. & Andersson, L.-O. (1977) *Biochem. Biophys. Acta* **490**, 104–112
- Pomerantz, M. W. & Owen, W. G. (1978) *Biochim. Biophys. Acta* **535**, 66–77
- Jordan, R. E., Oosta, G. M., Gardner, W. T. & Rosenberg, R. D. (1980) *J. Biol. Chem.* **255**, 10081–10090
- Griffith, M. J. (1982) *J. Biol. Chem.* **257**, 7360–7365
- Bjork, I. & Lindahl, U. (1982) *Mol. Cell. Biochem.* **48**, 161–182
- Lane, D. A., Denton, J., Flynn, A. M., Thunberg, L. & Lindahl, U. (1984) *Biochem. J.* **218**, 725–732
- Hurst, R. E., Poon, M. C. & Griffith, M. J. (1983) *J. Clin. Invest.* **72**, 1042–1045
- Griffith, M. J. & Marbet, G. A. (1983) *Biochem. Biophys. Res. Commun.* **112**, 663–670
- Tollefsen, D. M., Peacock, M. E. & Monafu, W. J. (1986) *J. Biol. Chem.* **261**, 8854–8858
- Akiyama, F., Seno, S. & Yoshida, K. (1982) *Tohoku J. Exp. Med.* **136**, 359–365
- Rosenberg, R. D., Oosa, G. M., Jordan, R. E. & Gardner, W. T. (1980) *Biochem. Biophys. Res. Commun.* **96**, 1200–1208
- Evington, J. R. N., Feldman, P. A., Luscombe, M. & Holbrook, J. J. (1986) *Biochem. Biophys. Acta* **871**, 85–92
- Seldin, D. C., Austen, K. F. & Stevens, R. L. (1985) *J. Biol. Chem.* **260**, 11131–11139

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