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Effects of different glycosaminoglycans on myosin ATPase activity in platelets

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Heparin is well known for increasing microvascular bleeding and capillary permeability [1]. Heparin also affects many functional characteristics of platelets, such as agonist-induced aggregation, platelet adhesion [2], release of serotonin, thromboglobulin and PF4 [3]. Furthermore, heparin induces thrombocytopenia [4], prolongs the bleeding time in patients and has antithrombotic activity [5].

Myosin is present in platelets [6], and its importance in the release reaction, clot retraction and interaction with actin is widely known [7]. Myosin–actin interaction produces kinetic energy, cytoplasmatic consistency and cellular protrusions as a consequence of G-actin quick polymerization [8]. Here we report studies performed on the ATPase activity of washed platelets in the presence of heparins having different molecular weights, native and desulfated dermatans and heparans. ATPase activity is determined in the presence of endogenous ATP.

Materials and Methods

Preparation of platelets. Blood was withdrawn from Sprague–Dawley male rats (300 g) (supplier: Nossan, Correzzana—Milan), usually fed on Altromin MT and tap water *ad lib.* Animals were housed in Makrolon cages and kept at constant temperature (22°).

The washed platelets (WP) were prepared as described by Sobel and Adelman [9]. Before the assay, platelets were lysed with 1:10 bidistilled water. Lysate was quickly centrifuged at 2500 *g* for 2 min and supernatant recovered and stored at 0°.

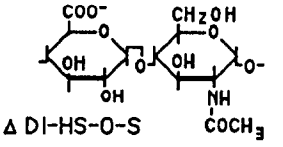
Myosin ATPase activity. Myosin ATPase activity was determined by luminometric procedure and calculated on Δ mVolts recorded on the first minute of reaction, and expressed as nmoles of hydrolysed ATP/min × 100 μL of lysate (2.5 pmol of ATP standard = 10 mVolts).

Glycosaminoglycans. Opocrin glycosaminoglycans purified from different sources, were dissolved in 0.9% NaCl saline solution. (a) Natural heparin from beef mucosa, molecular weight 10.6 kD; (b) low molecular weight heparins chemically degraded (2.9, 2.4, 1.7 and 1.3 kD); (c) heparan sulfate from beef spleen (23.3 kD, ±2.9 s.e.); (d) dermatan sulfate from pig mucosa (36.0 kD, ±3.0 s.e.);

Table 1

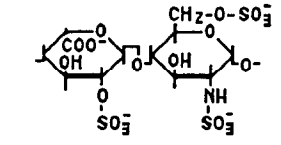
		RT	HEPARIN	HEPARAN
A	Δ DI-HS-O-S	3,61	5,0%	60,0%
B	Δ DI-HS-triS	28,20	60,0%	2,0%

A)



Δ DI-HS-O-S

B)

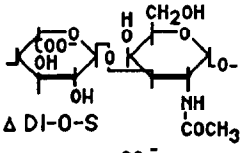


Δ DI-HS-triS

Table 2

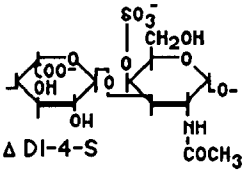
		RT	DS	DS des.
A	Δ DI-O-S	3,54	0,89%	64,07%
B	Δ DI-4-S	9,29	79,40%	7,69%
C	Δ DI-6-S	7,69	7,29%	0,38%
D	Δ DI-4,6-DiS	24,00	5,92%	0,00%
E	Δ DI-2,4-DiS	27,35	3,29%	0,00%

A)



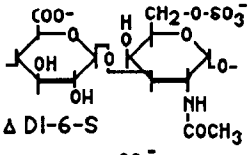
Δ DI-O-S

B)



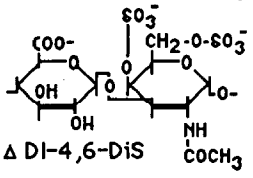
Δ DI-4-S

C)



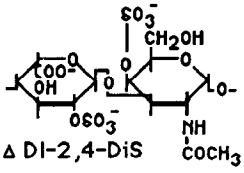
Δ DI-6-S

D)



Δ DI-4,6-DiS

E)



Δ DI-2,4-DiS

(e) chemically desulfated dermatan from beef mucosa (5.45 kD).

We tested also depolymerized heparin (with heparinase and heparitinase I) and depolymerized dermatan sulfate (with chondroitinase ABC). Enzymatic hydrolysis was performed according to Yoshida *et al.* [10]. The constitutive disaccharide profiles of enzymatically degraded heparin and heparan sulfate (Table 1), native and desulfated dermatan (Table 2) were measured by HPLC according to Linhardt *et al.* [11]. The retention times (RT) and percentage of disaccharides are reported.

Results and Discussion

Tests carried out in the presence of increasing concentrations of ouabain exclude the possibility of effects of glycosaminoglycans on Na^+-K^+-dependent ATPase activities.

The percentage of inhibition of myosin ATPase activity by different natural and modified glycosaminoglycans is reported in Table 3 which shows the effect of sulfate/carboxyl ratio.

ATPase activity is also inhibited in proportion to the increase in concentration of glycosaminoglycans (Fig. 1). The enzymatically depolymerized heparin (60% of trisulphated disaccharide) is an inhibitor of ATPase, while the enzymatically depolymerized dermatan sulfate (80% of disaccharide-4-S) does not inhibit this activity.

Natural heparan sulfate and dermatan sulfate are inhibitors of ATPase, but to a lesser extent than natural and low molecular weight heparin. Desulfated dermatan does not inhibit this enzyme. These highly polymerized compounds bearing ionic groups are able to affect ATPase activity. The percentage of inhibition increases in proportion to the content in sulfate groups per disaccharide (Fig. 2).

Heparin impairs the catalytic center of the ATPase. Tests carried out on purified myosin ATPase indicate a competitive inhibition (increase of K_m in presence of heparin without change of V_{max}). Therefore, glycosaminoglycans compete with ATP for the catalytic site. Sulfate groups of polysaccharides are very important in this interaction. ATP is known to interact on the catalytic site of ATPase by hydrogen bonds. One of these bonds links γ-phosphate

Table 3

Molecular weight (kD)	Sulfate/carboxyl ratio	% Inhibition of myosin ATPase*
Heparins		
10.6	2.05	45.0
2.9	1.95	47.5
2.4	1.95	42.0
1.7	2.03	49.0
1.3	2.00	47.0
Enzymatically degraded	—	38.0
Heparans		
23.3	0.88	25.0
23.3	0.95	25.0
Dermatans		
36.0	1.14	20.0
Enzymatically degraded	≈1.00	2.5
Desulfated dermatan*	≈0.00	5.0

* Twenty-five micrograms of glycosaminoglycans were tested as inhibitors in 600 μL final volume containing 100 μL of platelet lysate.

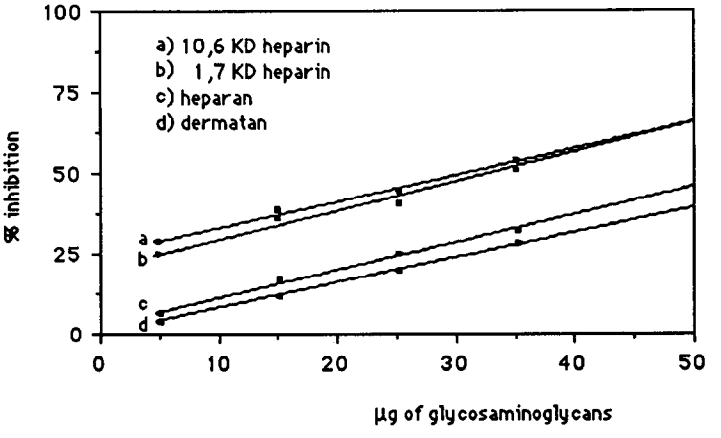


Fig. 1. Inhibition of myosin ATPase activity by increasing amounts of different natural and modified glycosaminoglycans. One hundred per cent myosin ATPase activity is referred to 0.9% NaCl incubated myosin.

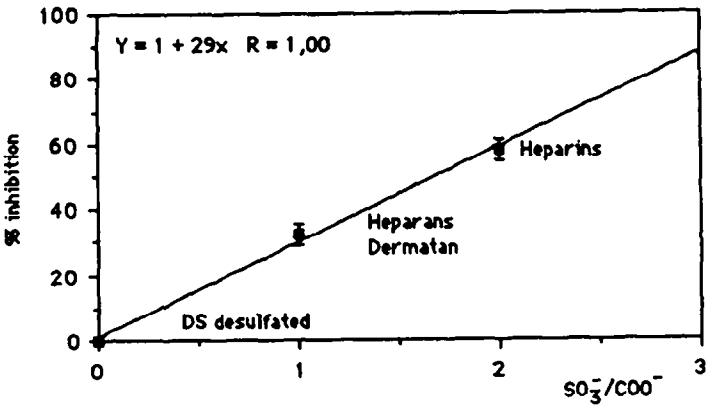


Fig. 2. Correlation between per cent inhibition with sulfate/carboxyl ratio of different sulfated and desulfated glycosaminoglycans.

and hydrogen of the hydroxyl groups of a threonin residue [12]. The same residue could interact with one of the sulfate group of the hexosamine belonging to disaccharide units. This interaction is likely involved in hindering the ATPase catalytic center to ATP. Studies on plasma rich in platelets and on washed platelets revealed evident heparin uptake by platelets, its metabolism and release [13]. Therefore heparin and its depolymerized product could probably interact with myosin ATPase of the platelet acto-myosin system.

Studies on vascular smooth muscle cells revealed the binding and internalization of heparin [14] and this vascular component is very important in haemostasis [15]. Besides, the promotion of bleeding heparin appear also to be related to the competitive inhibition of myosin ATPase activity on smooth muscle cells of vessel wall.

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