ANTICOAGULANT ABILITY OF HEPARIN FRACTIONS

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The anticoagulant ability of a heparin fraction containing four sulfuric acid residues per disaccharide structural unit of the macromolecule is 1.40 times greater than that of a fraction containing three sulfuric acid residues per unit.

KEY WORDS: blood coagulation; heparin and its fractions; anticoagulant ability.

Heparin preparations isolated from many tissues consist of a natural complex of fractions of this biopolymer that differ chiefly in their content of sulfuric acid residues [1-3]. The great diversity of the biological functions of heparin can be considered to be linked somehow with its natural chemical and physical heterogeneity [2, 4]. Quantitative investigations into this problem are considerably hampered by the difficulty of isolating individual fractions of this glycosaminoglycan [5].

The object of this investigation was to study the relative anticoagulant ability, i.e., the property of prolonging the blood clotting time, of homogeneous heparin fractions containing three (HP-3S) and four (HP-4S) sulfuric acid residues per repeating disaccharide structural unit of their macromolecule.

EXPERIMENTAL METHOD

HP-3S and HP-4S were obtained from heparin (Spofa, Czechoslovakia) as the potassium salts. The anticoagulant activity of HP-3S and HP-4S was compared entirely for fractions isolated from the same sample of the manufactured product. The time of delay of blood clotting was determined visually from the appearance of the first fibrin clots in experimental and control samples. In the experimental samples rabbit blood was mixed with heparin solution (in physiological saline) in the ratio of 2:1. In all samples the volume of the mixtures was 0.3 ml. Observations were made at 20°C.

TABLE 1. Ability of Heparin Fractions to Delay Blood Clotting

Expt. No.	Preparation No.*	No. of groups per disaccharide unit (A)	K_1	K_2	$\frac{K_2}{K_1}$	$\frac{K_1}{A}$	$\frac{K_2}{A}$
1	I	3,29	0,65	—	1,38	0,20	—
	II	4,04	—	0,90		—	0,22
	III	3,25	0,65	—	1,40	0,20	—
2	IV	3,92	—	0,92		—	0,23
	V	3,17	0,62	—	1,40	0,20	
3	VI	3,80	—	0,87		—	0,23
4	VII	2,97	0,60	—	—	0,20	—

*Results of analyses (in mmoles/g anion of biopolymer): in preparations I, III, V, and VII – glucosamine 1.32, hexuronic acids 1.43-1.54, sulfate groups 4.20-4.58; in preparations II, IV, and VI (in the same order) 1.40, 1.24-1.43, and 5.00-5.93, respectively. Nitrogen content in all preparations 1.20-1.23; ratio between content of hexuronic acids and glucosamine content is 1.

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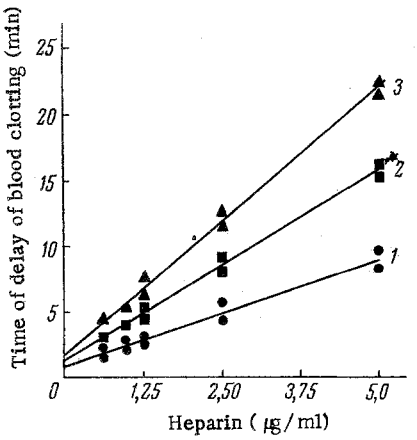

Fig. 1. Time of beginning of blood clotting as a function of concentration of heparin fractions: 1) original unfractionated preparation of heparin; 2,3) heparin fractions containing 3.25 and 3.92 sulfuric acid residues respectively per disaccharide unit.

EXPERIMENTAL RESULTS AND DISCUSSION

The time by which blood clotting was delayed was found to be a linear function of the heparin concentration over a wide range of values of the latter, as is clear from Fig. 1, showing the results for experiment 2 (Fig. 1, Table 1). All the other experiments gave similar results. The gradient of the straight line was greatest for HP-4S and that for HP-3S was much smaller. The smallest gradient of all was found on the graph for the original unfractionated heparin preparation. It follows that the blood clotting time can be expressed as $t = KC + a$, where K is the tangent of the angle of slope of the straight line to the abscissa, expressing the relationship between the time and heparin concentration (C), and a is a constant equal to the blood clotting time in the control test. The term K (determined graphically) is thus a value characterizing the anticoagulant activity of heparin. By comparing the values of K for different heparin preparations the relative ability (activity) of these preparations to delay blood clotting can be determined.

All preparations of fractions HP-3S and HF-4S isolated from different specimens of the manufactured product were characterized by values of K_1 and K_2 lying within the limits 0.60-0.65 and 0.87-0.90, respectively (Table 1). The ratio K_2/K_1 in every case was approximately 1.40, from which it follows that the anticoagulant activity of the HP-4S fraction was almost 1½ times greater than that of the HP-3S fraction. The activity of HP-3S was twice, and that of HP-4S four times greater than the activity of the original unfractionated heparing preparation (Fig. 1). The ratio of K_1 and K_2 to the content of sulfuric acid residues in each heparin fraction was 0.20 for HP-3S and 0.22-0.23 in HP-4S.

The results show that the anticoagulant activity of homogeneous heparin fractions depends on their content of sulfuric acid residues: fractions with a higher content of such residues have greater activity in this respect. In the heparin molecule one sulfuric acid residue is bound with an amino group of the glycosamine, whereas the others are bound by means of an ester bond with various carbon atoms of the same amino sugar and of hexuronic acid [1, 3]. Probably the role of each sulfuric acid residue differs in biochemical reactions. The different value of the ratios between K_1 and K_2, on the one hand, and the number of these sulfuric acid residues in HP-3S and HP-4S, on the other hand, may possibly be the result of the nonadditivity of each sulfate group as regards their participation in the reaction of inhibition of blood clotting. The assumption can be made that the activity of heparin in other biological processes in which it participates also depends to a certain extent on its content and disposition of its sulfuric acid residues, quite apart from the other chemical and physical features distinguishing the micromolecules of this biopolymer.

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