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Note on β-Heparin

IKUO YAMASHINA

Chemistry Department II, Karolinska Institutet, Stockholm, Sweden

The statement made by Marbet and Winterstein that β-heparin is a chemical entity has been confirmed by chemical and physicochemical analyses. The rate of acid hydrolysis and the optical rotation of the compound indicate that there is a resemblance between the internal structure of β-heparin and of chondroitin sulfuric acid. With respect to the anticoagulant activity, it is concluded that the mechanism of action of β-heparin differs in some respects from that of heparin and of heparin monosulfuric acid. The anticoagulant strength is found to be greatly dependent upon the nature of the coagulation system used for its assay.

In 1951 Marbet and Winterstein [1,2] reported the isolation of a new mucopolysaccharide from the lungs of some species of animals; it was named β-heparin. This β-heparin showed a resemblance to chondroitin sulfuric acid. It contained galactosamine and was levorotatory. Unlike chondroitin sulfuric acid, however, it exerted a relatively strong anticoagulant activity. Heparin and heparin monosulfuric acid, the only previously known natural anticoagulants of this type, contain glucosamine.

The present paper describes the results of an investigation carried out in order to establish the chemical entity of β-heparin.

Some evidence is also presented suggesting that the mode of the anticoagulant action of β-heparin may differ from that of the usual heparin.

Two samples of β-heparin, one from sheep lungs and one from ox lungs — which through the courtesy of Drs. Winterstein and Marbet, Hoffman-La Roche, Basel, had been given to professor Jorpes — were kindly put at my disposal for analysis. I was thereby enabled to confirm all the chemical findings of Marbet and Winterstein and to add some additional information in favor of the supposition that β-heparin is a chemical entity. With respect to the anticoagulant action of this form of chondroitin sulfuric acid, it was possible to show that the biological activity is greatly dependent upon the coagulation system in which it is measured.

EXPERIMENTAL

1. *Analyses.* We determined the moisture, ash, sulfur and acetyl contents and the optical rotation of the sheep β-heparin and confirmed the data reported by Marbet and Winterstein. For the acetyl determination, the method of Lemieux and Purves [3] was

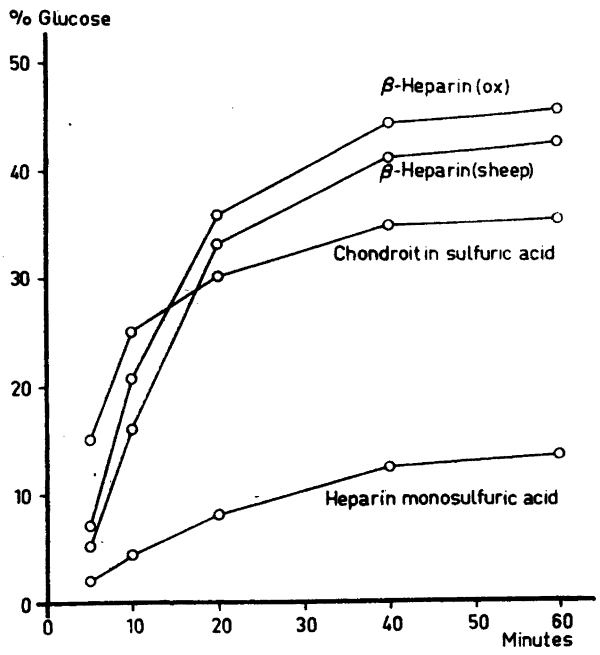

Fig. 1.

adopted instead of the methods of Viditz [4] and Kunz [5] used by the Swiss workers. The first-mentioned procedure has been found to be the most reliable for heparin [6]. The analysis of the single amino sugars was performed according to the ion exchange method developed by Gardell [7] in this laboratory. Heparin monosulfuric acid (prepared from ox lung with the method of Jorpes and Gardell [8]) was also analyzed as a reference substance. In accordance with the previous findings, heparin monosulfuric acid gave only D-glucosamine, although a rather low value, 27.5 %, was obtained as compared to the theoretical figure (42.8 %), possibly due to partial decomposition of the amino sugar during the preliminary hydrolysis. On the other hand, the β-heparin preparation was found to contain

Table 1. Anticoagulant activity of β-heparin, chondroitin sulfuric acid and heparin monosulfuric acid.

The figures represent the activity in international heparin units per mg water free substance. Samples 1 and 2 were analyzed with the thrombin method on three different days and sample 3 on two days.

	Studer and Winterstein [14]	Ox blood *In vitro*	British Pharmacopoeia 1953	U. S. P. XIV 1950	*In vivo*
1. β-Heparin (sheep)	50.4, 49.9; 56.3, 51.6; 49.1	3.4 4.2	0	<2	3 (cat) 3—4 (dog)
2. β-Heparin (ox)	24.6, 25.7; 24.3, 24.3;	8.3	3—4	<3	6—8 (cat) 8 (dog)
3. Heparin monosulfuric acid	7.7, 7.3;	4	<3	6	
4. Chondroitin sulfuric acid		0		0	

D-galactosamine as the only amino sugar component, the quantity found being 28.1 %. It may therefore be concluded that the anticoagulant activity of the preparation is not due to contamination with heparin.

The homogeneity of this preparation was then examined by electrophoresis on a slab of hyflo super-cel [9]. Electrophoresis was performed twice at pH 4.7 (0.1 *M* acetate buffer) for 8 and 12 hours, respectively. In both runs only one peak was observed.

The chemical and physical analyses described above seem to support the statement of Marbet and Winterstein that β-heparin is a chemical entity *per se*.

2. *Acid hydrolysis.* Several polysaccharides, such as heparin, heparin monosulfuric acid and hyaluronic acid, can be differentiated by comparing the rate of hydrolysis in 7.5 % (by volume) sulfuric acid at 100° C [10]. The β-heparin preparations (both from sheep and ox) were therefore hydrolyzed under the aforementioned conditions. The result was compared with those for chondroitin sulfuric acid, prepared from ox cartilage with the method of Strandberg [11] and for heparin monosulfuric acid. As can be seen from Fig. 1, the rate of hydrolysis of β-heparin closely resembled that of chondroitin sulfuric acid, suggesting a similarity in the internal structure of these two compounds.

3. *Anticoagulant activity.* Marbet and Winterstein measured the anticoagulant activity of β-heparin from ox lungs by the thrombin method and reported appreciably high values, about 36 international heparin units per mg. Although Blombäck *et al.*[12] recommend this method for heparin assay, it seemed of interest to ascertain whether the other assay methods would provide any different results. The anticoagulant activity of the β-heparin preparations, the chondroitin sulfuric acid and the heparin monosulfuric acid was therefore determined *in vitro*, using the four methods described in the paper of Blombäck *et al.* *. An *in vivo* determination was also carried out on a cat and a dog, the coagulation time being determined according to the technique of Bergqvist [13]. The results of these experiments are summarized in Table 1.

As can be seen in Table 1, the anticoagulant activity of the β-heparin preparation was remarkably low except with the thrombin method. The activity of heparin monosulfuric acid was fairly constant, irrespective of the method applied. The chondroitin sulfuric acid was inactive. It can be concluded from these findings that the mechanism of action of β-heparin differs in some respects from that of heparin and of heparin monosulfuric acid.

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