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Molecular Weight and Glycosamine Glycane Content of Raparin, a Heparinoid Obtained from *Rapana venosa* (Valenciennes 1846)

Rapana venosa (Valenciennes 1846)'dan Elde Edilen Heparinoid, Raparinin Molekül Ağırlığı ve Glikozamin Glikan İçeriği

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Abstract

In this work, raparin, a new heparinoid obtained from *Rapana venosa*, was investigated and its molecular weight and glycosamine glycanes content was determined. The molecular weight was found as 30000 Dalton. Glycosamine glycane amount of raparin corresponded to heparin as 468 mg/mg by Kuizenka and Spaulding and 0,308 mg/mg by Charles and Scott methods.

Key words: Raparin, gel permeation chromatography, glycosamine glycanes assay.

Introduction

Heparinoids are subtances showing heparin-like activities. They have anticoagulant, fibrinolytic and antiagregant activities. These agents have been used in the treatment of thromboembolic conditions as deep venous thrombosis and pulmonary embolism. Heparin is a sulfated polysaccharide which is synthesized as a proteoglycane by connective-tissue type mast cell, vascular endothelial cells, basement membrane and intestinal mucosa. Heparin which originated from animal sources was obtained from firstly the dog liver (McLean, 1916; Howell and Holt, 1918) and later from ox, pig, bowin and sheep.

In most tissues, the proteoglycane contains 10-15 polysaccharide chains (MV 60000 - 100000), depolymerized after biosynthesis by an endo-β-glucuronidase which thus gives rise to the fragments (MV 6000 - 25000). These compounds are used in medical therapie.

Heparins cover the aminosugars, glycosamine and galactosamine with the nitrogen substituted by sulfate or acetyl, uronic acids as glucuronic acid and iduronic acid. It is overall negatively charged long-chain polymer and has remarkable biological activities. The polysaccharide chains of heparin is composed of alternating units of hexuronic (D-glucuronic or iduronic) acid and D-glycosamine, mostly substituted by sulfate groups as ester.

Heparin-like anticoagulant activity has been demonstrated in marine organisms such as various algae (Güven *et al.*,1979), algal polysacharides (Güven *et al.*, 1991) and animals e.g. *Stichopus japonicus* (Fan *et al.*, 1980, 1983; Ruan *et al.*, 1986) and *Holothuria leucospilota* (Fan *et al.*, 1983; Wan *et al.*, 1985; Ruan *et al.*, 1986).

Another heparinoid was obtained from *Rapana venosa* (Valenciennes) (Gastropoda) collected from the Black Sea, near the Bosphorus(Güven *et al*; 1991). *R.venosa* originated in the Sea of Japan and first appeared in the Black Sea in 1946 at the mouth of Danube river and rapidly spread outwards (Drapkin, 1953). It was first detected in Turkish Black Sea coast in 1960 by

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Fisher-Piettee, (1960) and latter by Bilecik (1975). A heparin-like substance, raparin was first obtained from *R. venosa* by Güven *et al.*, (1991) and later it was fractionated by Genc *et al.*, (1996).
The other contents of *Rapana venosa* investigated were insulin (Akıncı *et al.*, 1998; Akıncı *et al.*, 1999), enzymes (Akıncı *et al.*, 1998), fatty acids and sterols (Guven *et al.*, 1999), vitamins D_2 and D_3 (Güven *et al.*, 2001).
This paper reports the determination of the molecular weight and content of glycosamine glycane of raparin.

Material and Methods

Rapana venosa was collected from the Black Sea near Bosphorus. Raparin was obtained from R.venosa through Charles and Scott (1933) (A) and Kuizenga-Spaulding (1943) (B) methods. It was fractionated on Sephadex G 50 column (2.8x70 cm) and eluted with distilled water, flow rate 50 ml/h. Elution curve was plotted for each 5 ml eluate at 190-200 nm (Genç *et al*; 1996). The fractions obtained were: (5-70 ml), and (170-280 ml) and lyophilized. Its molecular weight was determinated by HPLC-GPC (gel permeation chromatography). Detection was made at UV 205 nm. Glycosamine glycane was determinated by Muir method. Heparin was used as control in this assay.

Results and Discussion

The results of A and B in HPLC-GPC and refractometric analyses are shown in Figs.1and 2. As can be seen in the Figs. 1 and 2 the molecular weight of Fr.A is 30000 Dalton and the amount of glycosaminoglycanes is for (A) 0.308mg/mg and for (B) 0.469mg/mg (corresponding to heparin). The former is equvalent to 1/3 whereas the latter to ½ heparin.
The molecular weight of heparin varied 15000-30000 Dalton depending on its manufacturer and recently new products were prepared as 2500 Dalton, named low molecular weight heparin.
Mucopolysaccharides isolated from sea cucumber, *S.japonicus* and *H. leucospilota* contained galactosamine, glycuronic acid, fucose and sulfate groups in the approximate ratio 1:1:1:1:4 respectively. Their molecular weight was 30000 to 50000. The molecular weight and biological activity of raparin obtained from *R.. venosa* were found similar to those of heparinoids obtained from sea cucumber.
The activity of raparin is half of that of heparine. Biological effects of this polysaccharide of sea cucumber were also similar to those of heparin.
In earlier studies IR spectrum of raparin,showed 1647 cm^{-1} (C=O), 1232 and 1021 cm^{-1} (SO_4), 927 and 847 cm^{-1} (CO-S) bands similar to those of heparinoids (Genç *et al.*,1996).
In this work the results of glycosamine glycane supported that raparin was a heparin– like mucopolysaccharide

Özet

Bu çalışmada *Rapana venosa* (deniz salyangozu) dan elde edilen heparinoid bir madde olan raparin'in glikozamin glikan içeriği ile molekül tartısı bildirilmektedir. Kullanılan elde etme tekniğine göre raparin'in heparin ile eşdeğerliliği tayin edilmiştir. Raparin'in molekül tartısı HPLC-GPC metodu ile yapılan tayinde 30.000 Dalton olarak bulunmuştur. Diğer deniz canlıları *Stichopus japonicus* ve *Holothuria leucospilota*'dan izole edilen heparinoidlerin molekül tartısına uymaktadır.
Raparinin glikozamin glikan miktarı Charles ve Scott metoduna göre 0.308mg/mg ve Kuizenga-Spaulding metoduna göre elde etme de 0.469mg/mg heparine eşdeğer olduğu saptanmıştır. Bu bulgular evvelce antikoagulan aktivitesi ve IR spektrumu ile heparine benzerliği tespit edilen raparin'in bu yeni bulgular ile heparine benzer bir mukopolisakkarit olduğunu desteklemiştir.

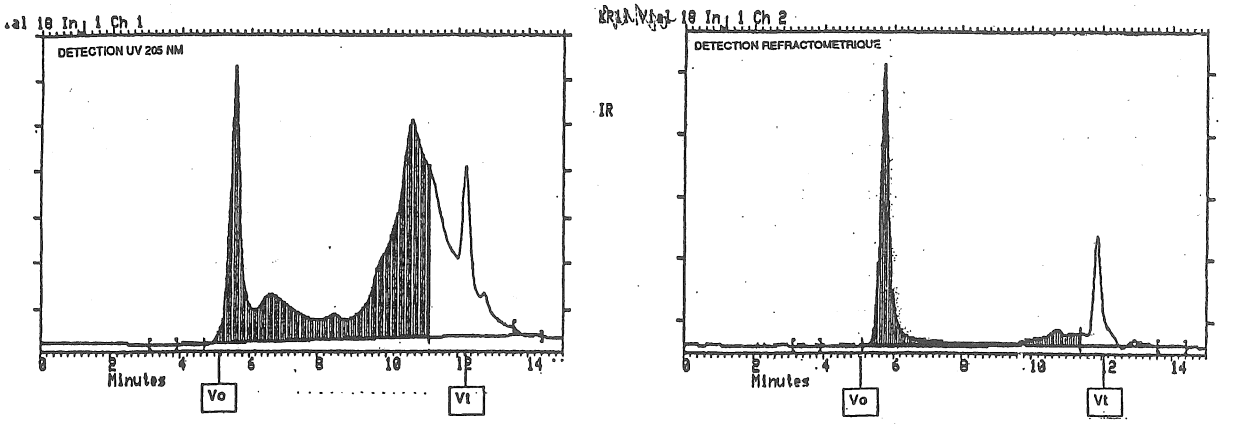

Fig.1.GPC chromatogram and refractometric detection of A

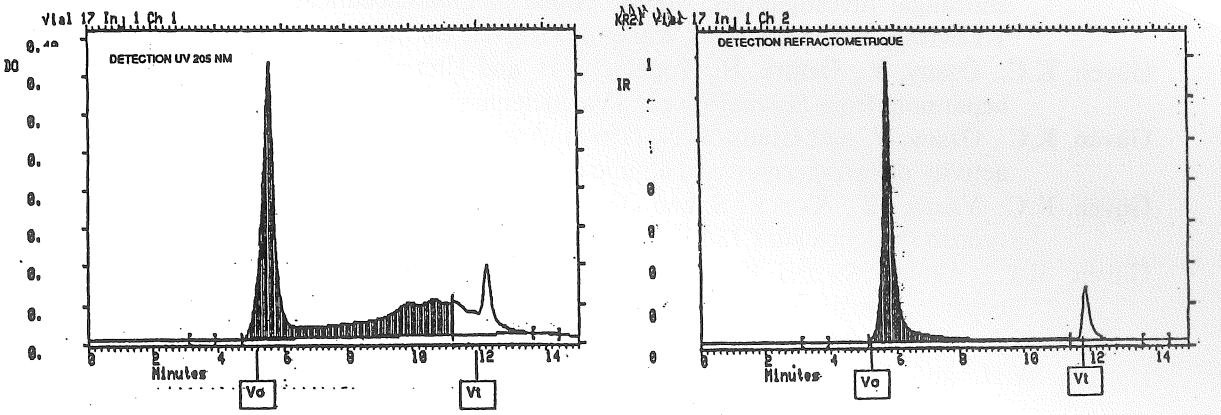

Fig.2.GPC chromatogram and refractometric detection of B

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