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Heparin like Compound from Green Alga *Chaetomorpha antennina* - As Potential Anticoagulant Agent

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Abstract: Anticoagulant properties of marine algae have been extensively studied for the last 60 years, among which green algae are considered as vital species with various bioactive substances. Heparin like compound was isolated from the seaweed *Chaetomorpha antennina*. The activity was studied following metachromatic dye method showing a yield of 2.3 mg/50 g. The anticoagulant activity of the extracted polysaccharide was assayed (in comparison with standard heparin) against the plasma protein thromoboplastin or thrombin of the sheep blood. In the agarose gel electrophoresis using acetate buffer, the band of the crude samples studied showed the band with similar mobility to standard heparin. The performance of the heparin extracted was fine and it resembles the commercial heparin to a greater extend and thus this species (*C. antennina*) could be claimed as alternative potential source for the heparin with anticoagulant activity.

Keywords: Heparin, agarose gel, metachromatic dye and anticoagulant activity

INTRODUCTION

There has been increasing interest in the systematic screening of bioactive compounds from natural resources such as marine organisms. Sulphated polysaccharides are a class of compounds having hemi-ester sulphate groups in their sugar units. In marine algae, they occur as sulphated fructose and sulphated galactans (Painter, 1983). These are commonly found in marine algae and higher animals, scarcely present in microbes and absence in higher plants. The highly diverse nature of sulphated polysaccharides from marine algae, the similarities between their structures with that of heparin and their anticoagulant nature were first reported by Chargaff *et al.* (1936). In recent years, sulphated polysaccharides from marine algae have been demonstrated to have many biological activities such as anticoagulant (Shanmugam and Mody, 2000).

Anticoagulant and antithrombotic activities are the most widely studied properties of sulphated polysaccharides. Anticoagulant activity of sulphated polysaccharides has been identified from several brown seaweeds such as *Padina gymnospora* (Silva *et al*., 2005), *Dictyota menstrualis* (Albuquerque *et al*., 2004), *Sargassum stenophyllum* (Duarte *et al*., 2001), *Spatoglossum schroederi* (Leite *et al*., 1998), the red seaweed *Gigartina skottsbergii* (Carlucci *et al*., 1997) and the green seaweed *Codium cylindricum* (Matsubara *et al*., 2001). Recent studies have revealed that some algal species contain repeated units in homofucans that have a large proportion of both α-1→3 and α-1→4 glycosidic linkages with sulphate groups at C-2 without excluding the presence of other sulphates, acetyl groups or branches at position 2, 3 or 4 (Chevolot *et al*., 1999; Patankar *et al*., 1993).

Heparin, the original unit was defined by Holwell (1922), as the amount of heparin which just prevents the clotting of 1ml of cat's blood for 24 h at 0ºC. The metachromatic activity of heparin fractions was dependent on sulphate content and high sulphate content of the product had a great metachromasia with Azure 'A' dye (Grant *et al*., 1984) and this method was used to determine the content of heparin like substance in animal tissues by Holick *et al*. (1985). Petitou and Van Boeckel (1992) stated that the antithrombotic effect of heparin enhanced inhibition of two of the coagulation factors viz., factor – Xa and thrombin.

In the present study this seaweed *Chaetomorpha antennina* has been screened for its sulphated polysaccharide activity intended for anticoagulant potential.

MATERIALS AND METHODS

The seaweed, *Chaetomorpha antennina* are collected from rocky shores of Veerampattinam, Pondicherry, Tamil Nadu in September, 2009. The collected seaweed was brought to CAS in Marine Biology, Annamalai University for further process. The seaweed were washed thoroughly with the seawater (to remove the epiphytes) followed by tap water and distilled water, in order to facilitate the removal of salts from the plants. The seaweeds were then shade dried, the extraction procedure of Holick *et al*. (1985) as modified by Subramanian

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(1998) to get better yield and activity was undertaken. In order to determine the heparin-like activity of polysaccharide fractions, metachromatic activity assay was conducted. The metachromatic dye, Azure-A has been used to determine calorimetrically the amount of heparin present in the samples. The color change in this case is from blue to violet. Reactions of this type have been used for the estimation of heparin (Grant *et al*., 1984). The United States Pharmacopoeia Method (1995) using sheep blood plasma was followed to study the anticoagulant activity. Electrophoresis was carried out in agarose gel plates. The gel plate was prepared with 0.6% agarose (2 to 4 mm thick) containing acetate buffer at pH 3.6 by the following method of Patel *et al*. (1980).

RESULTS

The amount of crude heparin and heparin like glycosaminoglycans was estimated as 46g/kg wet weight. The yield and activity of heparin and heparin like glycosaminoglycans in the experimental samples by metachromatic dye method were found to be 1080 IU/kg and 28 IU/mg respectively for *Chaetomorpha antennina* crude sample. In the United States Pharmacopoeia method, the yield and anticoagulant activity of *Chaetomorpha antennina* crude sample were reported to be 15.44 USP units/mg and 23.561USP units/kg of yield and anticoagulant activity respectively. Agarose gel electrophoresis of extracted heparin and heparin like glycosaminoglycans from *Chaetomorpha antennina* are presented in Fig. 1. As it can be observed that the agarose gel electrophoresis using acetate buffer, the band of the heparin like glycosaminoglycans in the crude sample studied showed the band similar mobility to heparin. The result of agarose gel electrophoresis showed the migration of crude sample as that of standard heparin. From this it could be inferred that the crude sample contains complex mixture of glycosaminoglycans.

Fig 1: Agarose gel showing similar mobility of crude extract and standard Heparin

DISCUSSION

Heparin is a highly sulphated glycosaminoglycan widely used as an injectable anticoagulant. It is also used to form an inner anticoagulant surface on various experimental and medical devices such as test tubes and renal dialysis machines. Pharmaceutical grade heparin is commonly derived from mucosal tissues of slaughter meat animals such as porcine intestine or bovine lung (Lindhardt and Gunay, 1999).

Earlier, Maeda *et al*. (1991) studied the anticoagulant activity of purified *Monostroma* polysaccharide thus obtained was estimated to be 5.9 times that of standard heparin. It showed a negative specific rotation ([α] D - 67.9) and was composed of major amounts of L-rhamnose (64.8%) and minor amounts of D-glucose (6.2%) and D-glucuronic acid (5.4%).

Screening of heparin-like compounds from organisms can be made by comparing the sample with the standard heparin by metachromatic-Azure 'A' assay for heparin activity (Subramanian, 1998). In the present study, initially, the seaweed extracts were compared with the standard heparin by their metachromasia behavior and their 'heparin' activity was confirmed anticoagulant activity.

Among the hot water soluble polysaccharides in the Chlorophyta examined, greater or lesser degrees of anticoagulant activities compared to that of heparin were identified in the samples. These activities were directly proportional to the amount of samples added below 10 µg for most of the algal polysaccharides. The high concentrations of heparin required to significantly inhibit compliment activation *in vivo* (Weiler *et al*., 1993) constitute an obstacle to heparin administration because of its anticoagulant activity.

Anticoagulant activity associated with polysaccharides in marine algae was first reported in *Iridaea laminariodes* by Chargaff *et al*. (1936) due to the presence of galactan sulphuric acid. After this, many seaweed species were reported to possess anticoagulant activity and they were *Grateloupia indica* (Sen *et al*., 1994) and *Grateloupia filicina* (Murugananthan, 2001). The present study has revealed the anticoagulant activity of *Chaetomorpha antennina* belonging to Chlorophyceae. Further, it could be noted from the earlier observations and from the present study that most of the chlorophycean members act as potential sources for anticoagulant activity. In the present study the total yield activity at the crude extracts of the dried seaweed extracts was 15.44 USP units/mg and 5,04,200USP units/kg respectively.

In the earlier study Murugananthan (2001) studied and the yield of activity was 7,02,000 units and 8,19,000 units per kg respectively in *G. filicina* and *G. lithophila*. No such yield report based on Azure-A assay is available in seaweeds for comparison. Further HPLC, FTIR and NMR studies needed for further comparison

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