

A LOW MOLECULAR WEIGHT HEPARIN FROM ASCIDIAN *PHALLUSIA NIGRA*
(CHORDATA: TUNICATA): PURIFICATION, STRUCTURE AND
ANTICOAGULANT ACTIVITY

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Heparin is a well-known glycosaminoglycan for its ability to prevent blood coagulation. The commercially preparation of heparin has been used clinically as anticoagulant. The appearance of bovine diseases and the haemorrhagic effect of high doses of heparin have limited its use. In this work, a heparin-like polysaccharide was extracted from *Phallusia nigra* and purified by using anion-exchange chromatography on a DEAE-cellulose and MONO-Q/FPLC columns. This glycan was identified by deamination with either nitrous acid and heparinase I. The polyacrylamide gel electrophoresis in barbital buffer showed that heparin from *P. nigra* has low molecular weight (~12 KDa). The anticoagulant activity was measured in the aPTT assay, in which it showed to be ~60-fold less anticoagulant (3 units/mg) than bovine heparin (180 units/mg). Ascidian heparin is able to inhibit the factor Xa via antithrombin ($IC_{50} = 3.14 \mu\text{g/mL}$), but the thrombin inhibition was very lower ($IC_{50} = 0.74 \mu\text{g/mL}$) in comparison with mammalian heparin ($IC_{50} = 0.143 \mu\text{g/mL}$ and $IC_{50} = 0.90 \text{ ng/mL}$, respectively). Taken together these results can point to a selective activity of this heparin as an inhibitor of the factor Xa. A more detailed study about the structure of this heparin is being performed to establish a correlation among the low molecular weight, disaccharide content and anticoagulant activity.

Key words: ascidian, anticoagulant, glycosaminoglycan, heparin

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