Optimization of Chemical Sulfation, Structural Characterization and Anticoagulant Activity of *Agaricus bisporus* Fucogalactan

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Introduction: There are many studies focused in evaluating possible anticoagulant activity of chemical sulfated polysaccharides, which are usually obtained using $ClSO_3H$ or SO_3-pyridine as sulfation agents. However, the conditions to obtain chemically sulfated polysaccharides are generally different for each study, and there is little information about obtaining optimal sulfation conditions to produce molecules with improved anticoagulant activity. **Objective:** The aim of this investigation was to obtain the optimal sulfation conditions of a fucogalactan to produce a molecule with greater anticoagulant activity. **Material and Methods:** The fucogalactan was extracted from *A. bisporus*. It was sulfated modifying reaction time, molar ratio of sulfation agent to hydroxyl group on the polysaccharide ($\eta ClSO_3H/OH$), and ratio of total reaction volume to weight of sample (V_T/w; $\mu L\ mg^{-1}$). Native and sulfated fucogalactans were characterized by methylation, NMR and HPSEC-MALLS analyses, and the anticoagulant activity was evaluated by APTT and PT. **Results and Discussion:** The fucogalactan (Mw=12,800 g mol^{-1}) presented a (1→6)-linked α-D-Gal*p* main-chain, partially methylated at O-3, and partially substituted at O-2 by non-reducing end-units of α-L-Fuc*p* or α-D-Gal*p*. The sulfated fucogalactan with the highest DS value (2.83) had the best anticoagulant activity. This sulfated fucogalactan (*Mw*=10,800 g mol^{-1}) was obtained with optimal conditions of $\eta ClSO_3H/OH$ of 18, V_T/w of 100, in 6 hours of reaction. It produces a linear increment of APTT for concentrations of 15 to 45 µg mL^{-1}, whereas PT was almost constant between 20 and 400 µg mL^{-1}, suggesting an action via inhibition of the intrinsic pathway of blood coagulation. NMR and methylation analyses showed that α-D-Gal*p* units of the main chain were greatly sulfated on 2-*O*-, 3-*O*-, and 4-*O*-positions. **Conclusion:** Though chemical sulfation of polysaccharides generally gives rise to anticoagulant molecules, a detailed evaluation of sulfation conditions is necessary when the goal is to obtain a greater activity.

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