

A Note on the Impact of Stabilization and Degradation of Deep-Sea Bacteria

Abstract

Polysaccharides are highly heat-sensitive macromolecules, so high temperature treatments are greatly destructive and cause considerable damage, such as a great decrease in both viscosity and molecular weight of the polymer. The technical feasibility of the production of exo polysaccharides by deep-sea bacteria *Vibrio diabolicus* and *Alteromonas infernus* was previously demonstrated using a bioproduct manufacturing process. The objective of this study was to determine which sterilization method, other than heat sterilization, was the most appropriate for these marine exo polysaccharides and was in accordance with bioprocess engineering requirements. The changes to both the physical and chemical properties of the sterilized exopolysaccharides were analysed. The use of ethylene oxide can be recommended for the sterilization of polysaccharides as a weak effect on both rheological and structural properties was observed. This low-temperature gas sterilizing process is very efficient, giving a good Sterility Assurance Level (SAL), and is also well suited to large-scale compound manufacturing in the pharmaceutical industry.

Keywords: Glycomimetic drug • Biofilm cells, Polydispersity • Cold plasmas

Introduction

Carbohydrates, particularly those found on cell surfaces, play critical biological roles, such as glycoconjugates or proteoglycans and especially their polysaccharidic chains called glycosaminoglycan's [1]. The discovery of the biological importance of these carbohydrates marked the beginning of glycobiology, glycolic, and carbohydrate-based drug discovery and glycomimetic drug development. Bacterial exopolysaccharides (EPS) serve distinct biological functions; one of the more important functions of EPS involves adhesion of cells to natural and artificial surfaces [2]. EPS immobilize biofilm cells and keep them in close proximity, thus allowing for intense interactions, including cell-cell communication. Biopolymers from marine prokaryotes offer significant structural diversity with novel material and biological properties. Biologically active glycopolymers derive their action from their molecular structure; including molecular size, polydispersity and repeating unit features varying in size, structure, linkages, and carried substituents. When sulfated, carbohydrates may be glycosaminoglycan-like components that exhibit many interesting properties with potential medical applications.

Description

Biologically active glycopolymers derive their action from their molecular structure; including molecular size, polydispersity and repeating unit features varying in size, structure, linkages, and carried substituents. When sulfated, carbohydrates may be glycosaminoglycan-like components that exhibit many interesting properties with potential medical applications [3]. Deep-sea hydrothermal vents discovered 30 years ago are a new source of a wide variety of fascinating microorganisms that are well-adapted to these extreme environments. The screening of a large number of isolates obtained

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from different oceanographic cruises led to the discovery of new species able to produce unusual EPS. Among them, the first species of *Vibrio* to be isolated from such an extreme environment was a mesophile *Vibrio diabolicus*, that secretes a linear EPS with a tetrasaccharide repeating unit, showing some resemblance to hyaluronic acid. This high-molecular-weight HE800 EPS (8×10^5 g/mol) secreted by *Vibrio diabolicus* exhibits interesting biological activities useful for increasing bone formation. HE800 EPS was evaluated on the restoration of bone integrity in an experimental animal model and was demonstrated to be a strong bone-healing substance without inducing any inflammatory reaction. As the sterilization process may affect the physical and chemical properties of the product and also induce loss of biological activity, finding suitable sterilization methods remains a challenge, especially for both heat and moisture sensitive molecules such as polysaccharides [4].

This data could suggest a discrepancy between the weak effect observed on rheological properties and the reduction in molecular weights for both EPSs, especially in the case of the EO treatment. This discrepancy could be explained by the concentrations of the EPSs in the rheological measurement and molecular weight determination methods (>1% w/w and 0.2% w/w, respectively) as they are not of the same range. In a concentrated polymer solution, both intra- and intermolecular interactions are higher than in diluted polymer solution. EO sterilization had a weaker impact than the other sterilization methods on the radius of gyration R_g , 44 and 86 nm for the treated HE800 and GY785 EPSs, respectively. Our data show that there is a molecular weight dependence of the radius of gyration [5]. Because the R_g is related to

the volume occupied by the molecule in a solution, the relationship between R_g and Mw suggests that the untreated and treated polysaccharides have similar structures or patterns.

The destructive effect of heating on polysaccharides is well described. Therefore in this study, we selected other sterilization methods such as ethylene oxide or EO, radiation by gamma and beta rays and cold plasmas, in order to evaluate the most appropriate sterilization procedure for the linear HE800 and branched GY785 EPSs. Sterilization efficiency was evaluated using a pour plate method. Every polysaccharide (0.2–0.4 g) was aseptically weighted, dissolved with 20 mL of sterile water and left at room temperature for 24 h for resuscitation and solubilization of polymers.

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Conflict of interest

No conflict of interest

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