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Surface-charged chitosan: Preparation and protein adsorption

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Abstract

Positive and negative charges were introduced to chitosan surfaces via methylation using methyl iodide (MeI) and reductive alkylation using 5-formyl-2-furan sulfonic acid (FFSA). Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS) and zeta potential measurement confirmed the presence of the desired functional groups on the surface-modified chitosan films. The chitosan films having negative charges of *N*-sulfofurfuryl groups on their surface (SFC films) exhibited selective protein adsorption against both negatively charged proteins (albumin and fibrinogen) and positively charged proteins (ribonuclease, lysozyme). Its adsorption can be explained in terms of electrostatic attraction and repulsion. In contrast, the adsorption behavior of chitosan films having positive charges of quaternary ammonium groups on their surface (QAC films) was anomalous. The quantity of the adsorbed protein tended to increase as a function of the swelling ratio of the QAC film regardless of the charge characteristics of the protein.

Keywords: Chitosan; Surface charge; Quaternary ammonium group; Sulfonated group; Protein adsorption

1. Introduction

Protein adsorption on material's surface is generally regarded as a primary event that occurs when the material comes into contact with biological surroundings. To a certain extent, such behavior reflects the performance of the material when it is used as biomedical devices or biomaterials. This type of "biofouling" is detrimental to technologies that require precise manipulation of proteins such as screening of DNA and protein libraries, controlling cell organization, and blood contacting devices. On the other hand, it is beneficial to some applications, for example, wound healing pads which demand plasma protein adsorption and subsequent platelet adhesion and activation. To address biofouling issues, much attention has been directed towards the development of chemical modification strategies that lead to material's surface having desirable biofouling characteristics.

Chitosan is a partially deacetylated form of chitin, a natural substance found abundantly in the exoskeletons of insects, shells of crustaceans, and fungal cell walls. Because of its favorable physicochemical and biological properties such as being biocompatible, non-toxic, and antibacterial, chitosan is considered as an attractive material that can potentially be used in many biomedical-related applications (Fu, Ji, Yuan, & Shen, 2005; Lee et al., 2002; Li, Liu, Liu, Liu, & Yao, 2005; Mi et al., 2001; Wang, Lin, Wang, & Hsieh, 2003; Yamamoto, Kuno, Sugimoto, Takeuchi, & Kawashima, 2005). The repeating units of chitosan are β-(1 → 4)-linked glucosamines that constitute a large number of hydroxy and amino groups. These two functional groups offer several possibilities for derivatiza-

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tion and immobilization of biologically active species (Ho et al., 2005; Lebouc, Dez, Desbrieres, Picton, & Madec, 2005; Liu, Zhang, Cao, Xu, & Yao, 2004).

Researchers have undertaken several routes to modify the surface of chitosan, the key purpose of which is to alter the chemical composition and the surface properties of chitosan to suit specific applications (Amiji, 1997; Chen, Kumar, Harris, Smith, & Payne, 2000; Justi, Favere, Laranjeira, Neves, & Casellato, 2005; Li, Liu, & Fang, 2003; Tsubokawa & Takayama, 2000; Wang, Fang, & Yan, 2001; Wang, Kao, & Hsieh, 2003; Zhu, Chian, Chan-Park, & Lee, 2005; Zhu, Wang, Yuan, & Shen, 2002; Zhu, Zhang, Wu, & Shen, 2002). Recently, we have reported work on chemical modification of the chitosan surface via reactions between the amino groups of chitosan and carboxylic acid derivatives (Tangpasuthadol, Pongchaisirikul, & Hoven, 2003) as well as PEG-functionalized aldehydes (Amornchai, Hoven, & Tangpasuthadol, 2004). The results from model studies suggested that amino groups on the surface of chitosan are reactive enough to react with a number of acid chlorides, acid anhydrides and aldehydes. Evidence from protein adsorption studies has confirmed the assumption that the surface hydrophobicity/hydrophilicity significantly influences protein adsorption (Amornchai et al., 2004; Tangpasuthadol et al., 2003).

Under acidic conditions, chitosan in its original form adopts a positive charge which can attract negatively charged plasma proteins leading to platelet adhesion and activation followed by thrombus formation and blood coagulation (Benesch & Tengvall, 2002; Okamoto et al., 2003). This sequential response should partly be responsible for the success of chitosan in wound healing acceleration (Azad, Sermsintham, Chandkrachang, & Stevens, 2004; Ishihara et al., 2003; Kojima, Okamoto, Miyatake, Kitamura, & Minami, 1998; Kojima et al., 2001). Such thrombogenic properties of chitosan cause serious failure when it is used in blood-contacting applications, however. In light of heparin's success in suppressing plasma protein adsorption, incorporating negative charges of sulfonate or sulfate groups has been proposed as an effective way to reduce the thrombogenic property of chitosan. It was revealed that blood compatibility of a chitosan surface can be improved by complexation-interpenetration methods using sulfonate derivative of poly(ethylene glycol) (Amiji, 1997), heparin and dextran sulfate (Amiji, 1996), and by direct sulfonation using sulfur trioxide–pyridine complex (Lin & Lin, 2001). Under homogeneous conditions, the reaction between the amino groups of chitosan and the sodium salt of 5-formyl-2-furansulfonic acid followed by reduction yielded *N*-sulfofurfuryl chitosan having non-thrombogenic properties (Amiji, 1998). Sulfation of chitosan by chlorosulfonic acid/DMF resulted in sulfated chitosan, which showed strong anticoagulant activity (Vongchan, Sajomsang, Subyen, & Kongtawelert, 2002).

Quaternary ammonium chitosan has attracted considerable interest because of its improved aqueous solubility and antimicrobial activity in a broader pH range in comparison with native chitosan (Jia, Shen, & Xu, 2001; Kim & Choi, 2002; Kim, Choi, Chun, & Choi, 1997). Antimicrobial activity is believed to originate from the ability of cationic species to bind with sialic acid in phospholipids, consequently restraining the movement of microbiological substances (Rabea, Badawy, Stevens, Smagghe, & Steurbaut, 2003). Oligomeric chitosan can also penetrate into the cells of micro-organisms and prevent the growth of cells by prohibiting the transformation DNA into RNA (Zheng & Zhu, 2003). Derivatives of quaternary ammonioum chitosan are typically synthesized either by direct quaternization of the amino groups of chitosan using alkyl halides under alkaline conditions (Curti, de Britto, & Campana, 2003; Domard, Gey, Rinaudo, & Terrassin, 1987), by the reductive *N*-alkylation reaction of chitosan with aldehydes via Schiff's base intermediates followed quaternization by methyl iodide (Jia et al., 2001; Kim & Choi, 2002; Kim et al., 1997), or by reductive *N*-alkylation reaction of chitosan with quaternary ammonium-type aldehydes (Suzuki, Oda, Shinobu, Saimoto, & Shigemasa, 2000). While the first two methods introduce alkyl groups not only to the amino groups, but also to the hydroxy groups, the last method is more selective to functionalization of amino groups. Reaction of amino groups of chitosan with glycidyl trimethylammonium was also suggested as an alternative to *N*-selective reaction (Lim & Hudson, 2004; Seong, Whang, & Ko, 2000).

Taking advantage of functional group availability for chemical reactions of the chitosan surface and the diversified bioactivity of its charged derivatives, this research aims to tailor protein adsorption of the chitosan surface by chemically introducing charged functionalities specifically to amino groups under heterogeneous conditions. The charged-modified chitosan surfaces are subjected to proteins having different molecular weights and isoelectric points. Besides hydrophobic interaction and hydrogen bonding, it is hypothesized that the extent of protein adsorption should depend also on electrostatic interaction between protein molecules and the modified groups on the chitosan surface. This surface modification should expand the applicability of chitosan in biomedical-related fields.

2. Materials and methods

2.1. Materials

Chitosan with DAC of 88% ($M_V = 645{,}535$ Da) was obtained from Seafresh Chitosan (Lab) Co., Ltd (Thailand). Methanol obtained as commercial grade was distilled over 4A molecular sieves prior to use. Methyl iodide (MeI), 5-formyl-2-furan-sulfonic acid (FFSA), sodium borohydride ($NaBH_4$), sodium hydroxide (NaOH) and sodium iodide (NaI) were purchased from Fluka (Switzerland) and used as received. Bovine serum albumin, fibrinogen, lysozyme, ribonuclease, Bicinchoninic acid assay kit