Journal of Fiber Bioengineering and Informatics 8:1 (2015) 57–67
doi:10.3993/jfbi03201506

Prospects of Silk Sericin Membranes Fabricated with Tyrosinase ⋆

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Abstract

Biological enzyme is a kind of substance, which can catalyze specific reaction. In our work, sericin membranes are prepared under the catalysis of tyrosinase, which promoting protein molecules crosslinking through tape casting method. Taking the water solubility of sericin membranes as the evaluation index, the optimal preparation conditions are determined as follows: the dosage of tyrosinase 1000 U/g, the reaction temperature 45 °C, for 90 min, 2% glycerol and drying temperature at 45 °C. The results of infrared spectra indicate that the structure of amide I is changed in crosslinked sericin membranes. The XPS results indicate the O atom content is increased in crosslinked sericin membrane. This verifies the crosslinking of sericin protein by tyrosinase.

Keywords: Tyrosinase; Crosslinking; Sericin Membranes; Water Solubility

1 Introduction

The last 20 years have seen an explosion in the level of research devoted to the development of new biodegradable materials, essentially due to the desire to protect the environment [1]. As a renewable resource, protein is abundant in nature, with non toxicity, non pollution, biodegradability and no stimulation, and has been a research hotspot in materials fields for a long time. As textile materials, the application of protein fibers such as silk and wool have been paid much attention in recent years. The protein collagen and gelatin were first studied as membranes materials. Numerous studies have investigated the effect of seeds plant protein on the preparation of edible films and food wrapping films. Orliac et al. [1] succeed to prepare sunflower protein membranes and also studied the effects of glycerol, ethylene glycol, diethylene glycol, triethylene glycol and propylene glycol on the thermoplastic potential of a sunflower protein isolate. Mengqin

⋆Project supported by National Natural Science Foundation of China (Nos. 51203107, 51373114); PAPD (A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions) and Qing Lan Project.

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L. et al. [2] prepared edible wheat gluten films with good comprehensive properties. Akturk et al. [3] studied the wound dressing membranes of sericin and collagen, which were stable in 4 weeks. Weijing L. et al. [4] studied the effect of different sugars grafting on physical properties of peanut protein films. In addition to those above seeds plant protein membranes, some other variety of protein membrane materials are also widely used in the cosmetics, food products, and medications.

Silk protein, including silk fibroin and sericin protein, is one of the earliest natural proteins used by people. Sericin, a kind of water-soluble globular protein, constitutes about 25% of silk protein [5]. Sericin is made of 18 amino acids such as serine, aspartic acid and tyrosinase. In the past, sericin was mostly discharged with the silk processing waste water. Recently, the researchers realized that the sericin protein represents a significant economic and social benefit if it can be recovered and recycled [5]. Consequently, sericin is used in the cosmetics, health care products, and medical fields. The sericin protein is easy to form membranes. The sericin membrane is usually used as cell culture medium and wound covering materials. However, the sericin membrane is thin and fragile. Some crosslinking agents are used to prepare crosslinking membrane, such as polyvinyl alcohol and polyethylene glycol. Ruijuan X. et al. [6] prepared sericin films with polyethylene glycol diglycidyl ether (PEG-DE) as crosslinking agent. The films showed good flexibility and high elongation at break. Su W. et al. [7] prepared blend hydrogel membranes with polyvinyl alcohol and sericin. On the positive side, chemical modification helps to form crosslinking between sericin and thereby enhance the properties of membranes. However, chemical modification may affect the biocompatibility of sericin protein and cause many toxicity problems.

On people advocating healthy and environmental materials, the development of green crosslinking agents such as enzymes become a new research hotspot. Enzyme as a biocatalyst with high substrate specificity, catalytic efficiency, biodegradability and non-toxicity, can catalyze the formation of inter- and/or intra-molecular crosslinks in sericin protein. Recently, tyrosinase was used as crosslinking agents in the preparation of sericin protein [8, 9], but the research on the properties of the sericin membranes was little.

Tyrosinases (EC 1.14.18.1) are copper-containing enzymes having a binuclear copper catalytic site [10]. They have been isolated from higher animals, microorganisms (bacteria, fungi), plants, and insects [11]. Tyrosinases are capable of oxidizing polyphenols to quinones. Thus, they are the rate-limiting enzyme required for melanin production [12]. The enzymatic reaction mechanism of tyrosinase involves first the formation of coordination bonds between Cu^{2+} and the amino residue of enzyme protein which become an active site with a specific three-dimensional structure. The substrate, contains *o*-quinone group, is capable to react with the active site due to proximity effects and orientation arrange. As a consequence, a composite product of enzyme and substrate is formed through hydrogen bonds between hydroxy of *o*-quinone and the residue of polypeptides. However, the composite product is unstable. The conformation of polypeptide become warped and the hydrogen bonds fracture in a short time. Then the composite product finally becomes *o*-quinone, which continues to occur series secondary oxidation reactions spontaneously. By dehydrogenation, the conformation of tyrosinase rotates and then restores the previous native conformation catalytic ability [13].

Our research was based on the mechanism in Fig. 1. Sericin was used as the substrate which contained phenolic hydroxyl. The tyrosinase catalyzed the sericin protein by two ways: one is oxidizing monohydric phenol of protein to diphenol (*o*-phenol, OPP) , the other one is generating