Available online at www.sciencedirect.com
ScienceDirect
Journal of Biotechnology 127 (2007) 508–519
Journal of BIOTECHNOLOGY
www.elsevier.com/locate/jbiotec

Tyrosinase-catalyzed grafting of sericin peptides onto chitosan and production of protein–polysaccharide bioconjugates

Anna Anghileri [a], Raija Lantto [b], Kristiina Kruus [b], Cristina Arosio [a], Giuliano Freddi [a,*]

[a] *Stazione Sperimentale per la Seta, via Giuseppe Colombo 83, Milano 20133, Italy*
[b] *VTT Technical Research Centre of Finland, P.O. Box 1000, FIN-02044 VTT, Finland*

Received 2 May 2006; received in revised form 7 July 2006; accepted 20 July 2006

Abstract

The capability of *Agaricus bisporus* tyrosinase to catalyze the oxidation of tyrosine residues of silk sericin was studied under homogeneous reaction conditions, by using sericin peptides purified from industrial wastewater as the substrate. Tyrosinase was able to oxidize about 57% of sericin-bound tyrosine residues. The reaction rate was higher than with silk fibroin, but lower than with other silk-derived model peptides, i.e. tryptic and chymotryptic soluble peptide fractions of silk fibroin, suggesting that the size and the molecular conformation of the substrate influenced the kinetics of the reaction. The concentration of tyrosine in oxidized sericin samples decreased gradually with increasing the enzyme-to-substrate ratio. The average molecular weight of sericin peptides significantly increased by oxidation, indicating that cross-linking occurred via self-condensation of *o*-quinones and/or coupling with the free amine groups of lysine and, probably, with sulfhydryl groups of cysteine. The high temperature shift of the main thermal transitions observed in the differential scanning calorimetry curves confirmed the formation of peptide species with higher molecular weight and higher thermal stability. Fourier transform-infrared spectra of oxidized sericin samples showed slight changes related to the loss of tyrosine and formation of oxidation products. Oxidized sericin peptides were able to undergo non-enzymatic coupling with chitosan. Infrared spectra provided clear evidence of the formation of sericin–chitosan bioconjugates under homogeneous reaction conditions. Spectral changes in the NH stretching region seem to support the formation of bioconjugates via the Michael addition mechanism.

Keywords: Silk sericin; Chitosan; Tyrosinase; HP-SEC; FT-IR; DSC

1. Introduction

Sericin is the glue protein that binds the two fibroin filaments as they are spun by the silkworm to form the cocoon. The term sericin identifies a family of proteins

* Corresponding author. Tel.: +39 02 2665990; fax: +39 02 2362788.
E-mail address: freddi@ssiseta.it (G. Freddi).

0168-1656/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2006.07.021

encoded by two genes, *Ser1* and *Ser2*, and produced through alternative splicing at the transcript level in the middle silk gland cells (Couble et al., 1987; Garel et al., 1997; Huang et al., 2003). Sericin proteins are characterized by an unusually high content of serine, which accounts for about 38 mol% (Takasu et al., 2002). The hydrogen bonding ability of hydroxyl amino acids is considered responsible for the glue-like properties of sericin, while a possible hypothesis for the heterogeneity of this group of proteins is related to the need of modulating viscosity and adhesiveness of the protein mixture as the cocoon is spun. Another important biological function that sericin is thought to perform is to lower the shear stress and to absorb the water squeezed from the stretched fibroin mass during cocoon spinning.

Sericin is usually removed from silk textiles before dyeing and finishing by a process called degumming, which takes advantage of the solubility of sericin in boiling aqueous solutions containing various degumming agents, such as soap, alkali, organic acids, or synthetic detergents, including proteolytic enzymes (Freddi et al., 2003). As a by-product of the degumming process, solubilized and partially hydrolyzed sericin peptides accumulate into the wastewater, with a substantial contribution to the total organic charge. This not only entails higher costs for wastewater treatment, but also leads to the loss of a still valuable protein material. Fabiani et al. (1996) developed a method for sericin recovery from wastewater by ultrafiltration. Recovered sericin peptides find effective application as ingredient of cosmetic formulations, including skin and hair care products, thanks to their moisturizing effect. However, in recent years the range of possible end-uses of sericin is considerably increasing (Zhang, 2002). Sericin was used as finishing agent for natural (Kongdee et al., 2005) or man-made textiles (Lee et al., 2004) with good results in terms of moisture absorption, antistatic properties, softness, and comfort. When air filters made of polyamide or polyester fibres were coated with sericin higher levels of antioxidant and antimicrobial activity were detected, suggesting their potential use as indoor air filters to reduce free radicals and fungi or bacteria contamination (Sarovart et al., 2003). Sericin can be cross-linked, copolymerized, or blended with other polymers to produce a new range of biodegradable materials with improved properties (Zhang, 2002; Cho et al., 2003; Ahn et al., 2001; Nagura et al., 2001). As a support for enzyme immobilization, sericin improved temperature stability of immobilized L-asparaginase (Zhang et al., 2004).

Biological properties of sericin have recently attracted a great deal of interest. In fact, Zhaorigetu et al. (2003a,b) reported data on the protective effect of sericin against both chemical- and UV radiation-induced tumorogenesis by reduction of oxidative stresses. Takeuchi et al. (2005) showed that high molecular weight sericin films effectively induced hydroxyapatite nucleation under biomimetic conditions. Sericin-coated α-tricalcium phosphate ceramics showed improved durability and desirable bioresorption rate as novel bone repair devices (Miyazaki et al., 2004). Tsubouchi et al. (2005) reported that sericin films enhanced attachment of cultured human skin fibroblasts. These findings seems to contrast with the hypothesis that sericin may be implicated in the immune response observed against virgin silk sutures (Soong and Kenyon, 1984). However, Panilaitis et al. (2003) showed that the biological response of sericin is probably dependent on the physical association with the fibres. Moreover, different sericin fractions may display different biological activities (Tsubouchi et al., 2005). Therefore sericin, either obtained directly from the cocoons or recovered from degumming wastewater, can be considered a valuable natural polymer worth of being used for a wide range of applications, including those related to biomaterials.

In an attempt to better exploit the properties of sericin proteins, we studied the possibility of using biotechnological tools to produce new bio-based, high-performing, and environmentally friendly polymers. Enzymes are expected to offer cleaner and safer alternatives to current chemical practices, owing to their specificity and selectivity which allows a more careful control of reaction conditions and final biopolymer structure by targeting selected reactive sites of the substrate (Shao et al., 1999). We specifically focused on the production of protein–polysaccharide bioconjugates via the tyrosinase-catalyzed oxidation-grafting reaction, according to a reaction scheme recently developed for the silk fibroin–chitosan polymer system (Sampaio et al., 2005; Freddi et al., 2006). Tyrosinase is a copper-containing enzyme widely distributed in nature, which has proved to be useful