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Dialdehyde cellulose-crosslinked collagen and its physicochemical properties

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ABSTRACT

The research has focused on the modification of the physicochemical properties of silver carp collagen by using 2,3-dialdehyde cellulose (DAC) as a crosslinking agent. DAC was prepared by a regioselective oxidation of cellulose with periodate. The effect of collagen crosslinking on its properties, particularly structural, biological, thermal and mechanical viscoelastic behavior has been investigated. FTIR studies demonstrate that crosslinking is achieved through the reaction of the DAC aldehyde groups with the free amino groups of collagen. Differential scanning calorimetry (DSC) measurements reveal that the denaturation temperature (T_d) of collagen after the modification with DAC increases from 79 to 94 °C. Moreover, the dynamic mechanical thermal analysis (DMTA) shows that the DAC crosslinking influences the viscoelastic behavior of fish collagen. Tan δ_{max} peak associated with the process of decomposition shifts toward higher temperatures, indicating a higher thermal stability of crosslinked collagen. Enzymatic analyses and assay of the crosslinking efficiency also demonstrate the stabilizing effect of DAC on collagen macromolecules.

1. Introduction

Collagen is the primary structural material of vertebrates and is the most abundant mammalian protein. It can be found in all animal parts, but it is mainly concentrated in skin-associated tissues and bones. The versatility of collagen as a building material is principally due to its unique properties and complex hierarchical structure. The basic raw materials for collagen isolation are pig and cow skin. However, at present collagen from alternative sources such as marine animals has gained attention. The fish skin is easily accessible, is not associated with the risk of outbreak of bovine spongiform encephalopathy, and it is not prohibited for religious reasons, and therefore, it constitute safe and attractive alternate source of collagen to mammals skin. In the literature, many papers are devoted to fish collagen. Unfortunately, the most of them concern the research on its preparation method and physicochemical properties [1–5], and only a few refer to modification and improvement of its properties [6–8]. The above issue is very important, especially in terms of the potential application of fish collagen on industrial scale to produce cosmetic and pharmaceutical articles.

The main aim of this study is modification of collagen from fresh skin of silver carp. Despite considerable effort devoted to research in the extraction procedures effect on solution of silver carp collagen and its physicochemical properties [9–12], significant areas remain poorly understood without knowledge of modification of its properties. Silver carp (*Hypophthalmichthys molitrix*) is a fresh water fish, which lives in a subtropical climate and collagen derived from its skin has, therefore, a temperature of denaturation (T_d = 34 °C) slightly lower than the T_d of bovine collagen [9,13]. On this basis, it is hypothesized that fish collagen is potential alternative to replace mammalian collagen. However, as shown in previous work [14,15], silver carp collagen exhibits relatively poor thermo-mechanical properties and fast biodegradation rate and therefore, must be submitted to crosslinking process. Generally, methods applied to stabilize various types of collagen include chemical crosslinking and/or physical treatments (dehydrothermal, radiation). Chemical reactions are favored by the large number of collagen functional side groups. The most common crosslinking means include glutaraldehyde, glyoxal, carbodiimide, transglutaminase and genipin. Recently, the dialdehydes following polysaccharides: cellulose [16–21], schizophyllan [22], starch [23], alginate [24] and xanthan gum [25] have received a great deal of attention as perfect protein crosslinkers. Modified polysaccharides were proved to be eco- acceptable [22]. It is worth noticing that dialdehyde cellulose (DAC) is biodegradable and biocompatible and

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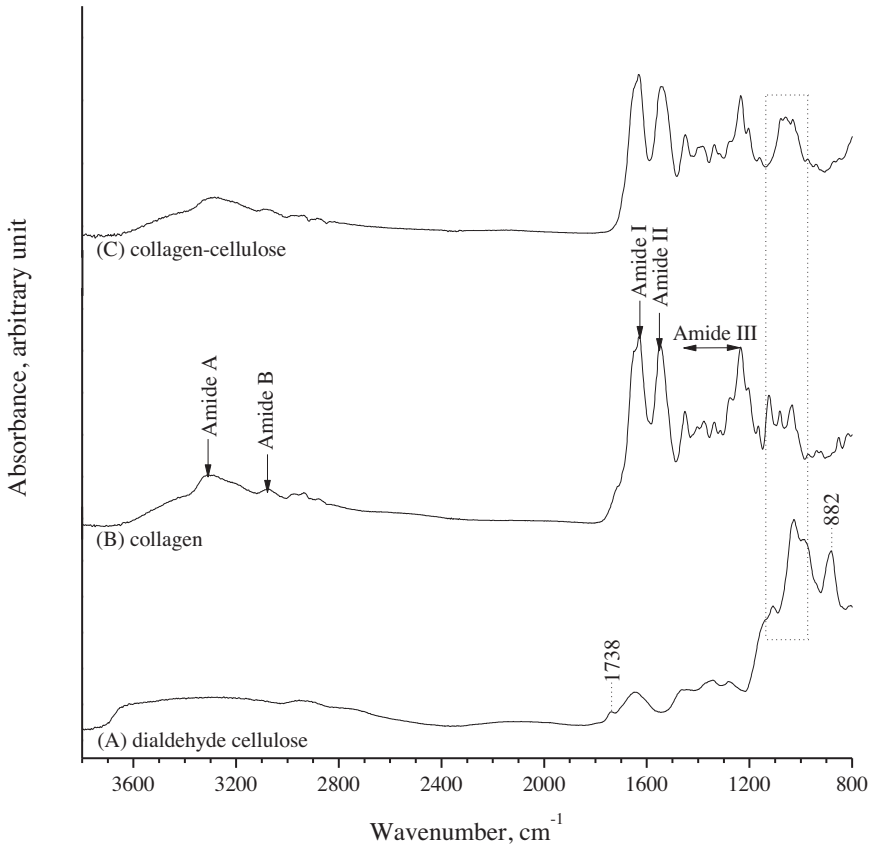

Fig. 1. FTIR-ATR spectra for samples: (A) 2,3-dialdehyde cellulose, (B) uncrosslinked collagen and (C) collagen crosslinked by 2,3-dialdehyde cellulose.

possesses a great potential in high-applications. The research outlined by Kanth et al. revealed that DAC brings about a significant increase in hydrothermal shrinkage temperature of type I collagen from rat tail tendon [16]. Additionally, the results presented by Lu et al. [19] showed that composite aerogels based on DAC and bovine collagen exhibit good biocompatibility. As it is reported [20] the incorporation of dialdehyde carboxymethyl cellulose (DCMC) to bovine collagen cryogels significantly improves the thermal stability and swelling rate of crosslinked collagen cryogels. Moreover, the coupling DCMC with bovine gelatin enhances thermal stability, mechanical properties and the barrier properties against UV light and water vapor of gelatin-DCMC films [18].

The growing importance and the growing number of possibilities to use of fish collagen cause that it would be worthwhile to assess the effect of crosslinking with DAC on its physicochemical properties and understand the mechanism of its stability against degradation and heat. To date, papers dealing with modifying of fish collagen are still limited. Moreover, there is no data on silver carp collagen modification. The research is mainly devoted to collagen from fish, such as chum salmon (*Oncorhynchus keta*) [6], catla (*Catla catla*), rohu (*Labeo Rohita*) [7] and tilapia (*Oreochromis niloticas*) [8] and characteristics of their biological and biochemical properties. The effect of temperature on the mechanical properties of modified fish collagen is not clearly elucidated.

In this paper, an attempt has been made to the synthesis DAC crosslinked silver carp collagen. The effects of crosslinking on collagen thermal stability and its rheological behavior have been investigated. The results of the temperature denaturation (T_d) and the temperature dependence of the dynamic mechanical thermal properties (DMTA) of collagen were discussed. The structure, porosity and crosslinking efficiency of crosslinked collagen have also been examined.

2. Materials and methods

2.1. Materials

The collagen type I was extracted from fresh skin of silver carp (*Hypophthalmichthys molitrix*) and supplied by AAG Sp. z o.o. (Poland). Some properties of collagen like: temperature of denaturation (T_d = 34.5 °C), amino acid composition, pH 3.2, and water content (91.5%), have been characterized in previous investigations. The concentration of collagen in the solution was established by estimating hydroxyproline using method recommended by the Standard number PN-ISO 3496:2000.

Collagenase from *Clostridium histolyticum* (type I, 0.25–1.0 FALGPA units/mg, ≥125CDU/mg), microcrystalline cellulose, sodium (meta) periodate, t-butyl alcohol, 4-(dimethylamino) benzaldehyde, hydroxyproline and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals of analytical grade were obtained from POCh-Gliwice (Poland) and used as received.

2.2. Preparation of 2,3-dialdehyde cellulose (DAC)

The DAC was obtained by periodate oxidation of cellulose by the method given by Kim et al. [26]. Briefly, microcrystalline cellulose (~10 g) was hydrolyzed in 5N hydrochloric acid (10 h, T = 85 °C). Hydrolyzed cellulose was then oxidized by sodium periodate (16.5 g) for 48 h, at 35 °C, pH 4.0 and preserved away from light. After this step the remaining periodate was decomposed by adding of excess t-butyl alcohol, and the reaction product was extracted with centrifugation (5000 × g, 15 min, 25 °C) until all iodic compounds were removed, and then dried at 35 °C. The periodate consumption was estimated by absorbance at 290 nm. 1% (w/v)