Carbohydrate Polymers 103 (2014) 250–260

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Elucidation of hydration dynamics of locust bean gum–collagen composites by impedance and thermoporometry

Ivy Kanungo, Nishter Nishad Fathima, Raghava Rao Jonnalagadda*, Balachandran Unni Nair

Chemical Laboratory, Central Leather Research Institute, Council of Scientific and Industrial Research, Adyar, Chennai 600020, India

ARTICLE INFO

Article history:
Received 29 August 2013
Received in revised form 9 December 2013
Accepted 14 December 2013
Available online 25 December 2013

Keywords:
Collagen
Locust bean gum
Impedance
Thermoporometry
Hydration

ABSTRACT

The intricacy of the different parameters involved in the hydration dynamics of collagen influences its performance as biomaterials. This work presents the molecular motions of collagen originating from the solvents and locust bean gum (LBG), which reveal the changes in solvation dynamics of the biopolymers affecting the surface as well as interfacial properties. Water, as a probe liquid bound in collagen has been investigated using a combination of thermoporometry, ATR-FTIR, circular dichroic spectroscopy, dielectric spectroscopy and SEM to explore the influence of LBG on collagen with respect to static and dynamic behaviour. The relaxation process of collagen in the frequency range of 0.01 Hz to 10^5 Hz and thermoporometry results indicate that the interfacial hydration dynamics are dependent on the applied concentration of LBG. This investigation explicitly reflects the rearrangements of the structural water clusters around the charged amino acids of collagen. These results can be employed to redesign the approach towards the development of collagen based biomaterials.

1. Introduction

Polysaccharides, the natural polymeric materials, have diverse set of functions. They play a well-known function as a reserved material in membranes utilized during germination (Prajapatia, Jani, Moradiya, Randeria, & Nagar 2013) and intracellular communication, while proteins function as structural materials and catalysis (Dionisio & Grenha, 2012). The current trend in the tissue engineering application is to mimic nature, leading to exploration of natural biopolymer in the biomedical and biopharmaceutical applications. The notion of polysaccharides is equally interesting in the design of nanostructured biomaterials (Rinaudo, 2008).

Locust bean gum (LBG), a non-starch natural polysaccharide consisting of galactose and mannose in the ratio 1:4 (Structure given as supplementary data Fig. S1), has a wide application in tissue engineering and pharmaceuticals due to their tailorable physico-chemical properties (Parvathy, Susheelamma, Tharanathan, & Gaonkar, 2005; Prajapatia et al., 2013). LBG is highly viscous, nonionic polymer, which is unaffected by variations in pH, salinity and temperature (Kaity & Ghosh, 2013; Kaity, Isaac, Mahesh Kumar, et al., 2013). The relative hydrophobicity of mannose permits the formation of strong intra-molecular hydrogen bonds, having a propensity to form aggregates in cold water due to the reduction of hydration of gums. The strong synergistic interaction of LBG with other polysaccharides is attributed to the numerous —OH groups, which results in the gelling structure for biopharmaceutical applications (Dakia, Wathelet, & Paquot, 2007; Kaity, Isaac, & Ghosh, 2013; Wang & Somasundaran, 2007).

Collagen based biomimetic scaffolds have made a great strides in the field of tissue engineering. Fibrillary collagen, one of the most abundant structural proteins present in the extracellular matrix (ECM), permits the incorporation of known biological signals recognized by the receptors and other intracellular proteins. A number of important aspects that determines the performance of biomimetic scaffolds include the composition, structure and mode of processing performed. Biomaterials designed to have specific dimensions, interfacial structure, hydration dynamics, interconnectivity and surface chemistry could in principle emulate the architectural features and cell signalling machinery of the natural extracellular matrices to promote regenerative medicinal application. Presence of higher polar groups such as carboxyl, hydroxyl or amine groups results in more polarity and higher wettability. Due to this fact, the controlled modification of polarity, hydrophobicity and charge becomes a complicated process for intended biomedical application (Desmet et al., 2009). The understanding of the interfacial or surface structure of the biomaterials and its relation to the macroscopic properties has proven to be an extremely valuable tool to comprehend the cell adhesion mechanism at molecular level. The orientation of water bridges influences the short and long range intermolecular interactions. The energetic contribution of

* Corresponding author. Tel.: +91 44 24411630; fax: +91 44 24911589.
E-mail addresses: clrichem@mailcity.com, jrrao@clri.res.in (J. Raghava Rao).

0144-8617/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.12.051

attractive hydration forces arising from water bridges is an interesting but often neglected aspect of macromolecular interactions. The hydration dynamics of protein is greatly influenced by the co-solutes such as cross-linkers (Farrist, Song, & Huang, 2009). Genipin, extracted from *Gardenia jasminoides* Ellis, is one of the crosslinking agents for proteins and polysaccharides (O'Brien, Harley, Yannas, & Gibson, 2005). Glutaraldehyde is the most widely used cross-linker for stabilizing collagen, but of recently, several reports demonstrate that genipin is about 10,000 times less cytotoxic compared to glutaraldehyde and is also biocompatible (Nishi, Nakajima, & Ikada, 1995). Crosslinking of collagen with genipin involves nucleophilic attack by primary amine groups of lysine or hydroxylysine amino acids residues of the polypeptide chains at the C3 carbon atom in genipin (Yoo, Kim, Kim, & Choi, 2011). Thereafter, Schiff base is formed leading to subsequent reactions that may be involved in the intramolecular and intermolecular crosslinking of collagen.

This work focuses on the understanding the hydration dynamics and designing of collagen–locust bean gum based composites crosslinked with genipin. As represented in Fig. 1, the designer materials facilitate multiple noncovalent as well as covalent interactions resulting in modulation of hydration dynamics of collagen. Dielectric techniques (Kandamchira, Kanungo, & Fathima, 2012) have been previously used to study the effect of water and electric field frequencies on the dielectric properties of constituent phases of collagen. Impedance measurement gives the molecular insights into the dipole–dipole interaction and hydration dynamics at the collagen–additives interfaces (Friess & Lee, 1996; Manikoth, Kanungo, Fathima, & Rao, 2012; Marzec & Warchol, 2005; Samouillan, Lamure, & Lacabanne, 2000; Samouillan, Lamureet al., 2000). Thermoporometry utilizes the traditional differential scanning calorimetric technique in an untraditional way to quantify water in nanoscale pores (Fathima, Baias, Blumich, & Ramasami, 2010; Fathima, Pradeepkumar, Rao, & Nair, 2010). In view of the above, the structural characteristics of water sorbed in the pores of the collagen–LBG composites are investigated using dielectric measurements, thermoporometry, circular dichroic spectroscopy, ATR-FTIR spectroscopy for the development of new biomaterials.

2. Experimental

2.1. Materials

Frozen rat tail tendon (RTT), excised from 6 month old albino rats (Wistar strain) were thawed and teased out. Locust bean gum (M_w ~310 kDa), genipin and picrylsulfonic acid [2,4,6-trinitrobenzene sulfonic acid (TNBS)] were purchased from Sigma–Aldrich. Water used for these studies was of millipore grade.

2.2. Isolation and collagen type I solution preparation

Tails of 6 month old male albino rats (Wistar strain) were excised due to its high purity, available lysine residues suitable for crosslinking and frozen at 253 K. The collagen type I solution was prepared after washing the teased collagen fibre with 0.9% NaCl at 277 K by acetic acid extraction method and salting out with NaCl (Chandrakasan, Torchia, & Piez, 1976). SDS-PAGE technique was employed to estimate the purity of extracted collagen. The collagen concentration in the solution was determined from the hydroxyproline content according to the method of Woessner (1961). A factor of 7.2 was used for calculating the mass ratio of collagen to hydroxyproline. The average molecular weight of

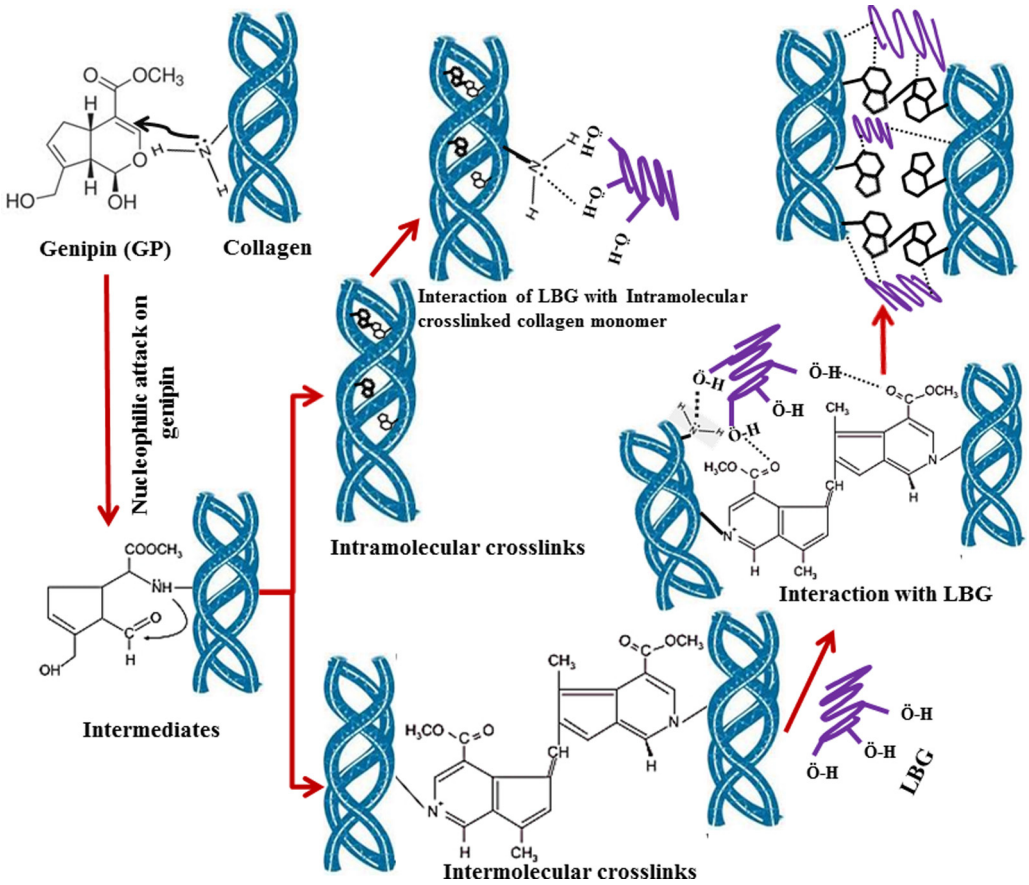

Fig. 1. Schematic representation of interaction of genipin crosslinked collagen–locust bean gum (LBG) composites interconnected with intra- and intermolecular hydrogen bonded network.