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Stabilization of collagen using plant polyphenol: Role of catechin

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Abstract

Collagen, a unique connective tissue protein finds extensive application as biocompatible biomaterial in wound healing, as drug carriers, cosmetics, etc. A work has been undertaken to study the stabilization of type I collagen using the plant polyphenol catechin. Catechin treated collagen fibres showed a shrinkage temperature around 70 °C implying that catechin is able to impart thermal stability to collagen. Catechin treated collagen fibres has been found to be stable even after treatment with high concentration of the secondary structural destabilizer, urea. Circular dichroism studies revealed that there is no major alteration in the structure of collagen on treatment with catechin. The study has demonstrated the involvement of hydrogen bonding and hydrophobic interactions as the major forces involved in the stabilization of collagen by the plant polyphenol, catechin.

Keywords: Collagen; Catechin; Polyphenol; Rat tail tendon (RTT); Tannin

1. Introduction

Vegetable tannins are an important species of compounds, which have extensive applications in the field of pharmaceutical, food processing and tanning industries. Vegetable tannins are water-soluble polyphenols that are present in many plants, which are also utilized as food [1]. The physiological roles of tannins and related polyphenols in plants have not yet been clarified, but their ability to form complexes with proteins or related biopolymers has been correlated with some protection of the plants from predators such as animals, insects and microbes.

Chemically tannins are mixture of several molecular species [2]. Vegetable tannins are classified as ester derived hydrolysable and flavanoid derived condensed tannins [3]. They are known to form complexes with proteins through multiple interactions [4,5]. Reactivity of condensed tannins with biomolecules is important due to their nutritional and physiological effects. Condensed tannins are polymerized products of flavanols and they are also referred as proanthocyanidins. Catechin, gallocatechin and epi-gallo-catechin are all precursors of condensed tannins.

Pharmacological properties of tannins have been investigated based on recent advances in the structural study of tannins in medicinal plants and various properties of tannins including antitumor and antiviral effects have been revealed [6]. Chung et al. had shown that tannins and their precursors can be beneficial as well as detrimental to human health depending upon the concentration of the exposure [7]. These effects are also attributed to the interactions of tannins with certain biomolecules in organisms. Most of these properties are dependent on the chemical structures or molecular shapes of tannins. Therefore, clarification of the molecular conformations of tannins and related polyphenols is a requisite to understand the process of molecular interaction of tannins with the biomolecules. In this work, we are presenting our study on the interaction of catechin, a condensed tannin precursor, with collagen.

The most abundant protein found in mammalian tissues is type 1 collagen. It is the main structural protein of skin, bone and tendon [8,9]. Collagen as a protein has a distinguishing feature, each molecule has a coiled coil structure with three polypeptide chains, two $\alpha_1(1)$ and one $\alpha_2(1)$ chain, wound together to form a right handed triple helix of about 3000 Å length and 15 Å diameter [11]. Earlier the ultrastructure of collagen has been well elucidated [10–12]. Collagen is an important biomaterial finding several applications as prosthesis, artificial tissue, drug carrier, cosmetics and in wound healing. In all these applications

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stable collagen is required and molecules used for stabilization should also be biocompatible. Earlier the plant polyphenols have been shown to stabilize collagen, which can find applications as a biomaterial [13,14].

Catechin contains flavon-3-ol structure. High concentrations of flavan-3-ols are found in green tea and wine [15,16]. Catechin has been shown to stabilize collagen against the attack of collagenase [17,18]. This work focuses in understanding the molecular level mechanism on the interaction of collagen and catechin. The biomaterial developed by stabilizing collagen using catechin with specific properties has great potential for the development of a new generation prosthetic implants.

2. Materials and methods

Catechin and bacterial collagenase type IA have been obtained from Sigma Chemicals Co., USA and used without further purification. Other chemicals used in the experiments are of analytical grade sourced from SD Fine Chemicals, India.

2.1. Sample preparation

Collagen fibres have been teased out from tails of 6 months old male albino rats (Wistar strain) and thoroughly washed and stored at −20 °C until needed.

2.2. Investigation of the hydrothermal stability

Teased collagen fibres have been washed with 0.9% NaCl at 4 °C, to remove the adhering muscles and other soluble proteins. The rat tail tendon (RTT) collagen fibres have been washed extensively in double distilled water at 4 °C.

RTT fibres have been treated with different concentrations of catechin ranging from 0.5 mM to 0.02 M for 24 h at room temperature (27 °C) without any agitation. The hydrothermal stability/shrinkage temperature of the treated fibres has been determined by the standard method [19]. A small strip of fibre has been cut and placed on a grooved microscopic slide containing water. The slide in turn has been placed on a heating stage along with a microscope mounted above the heating stage (metal stage with nichrome winding). The rate of heating has been controlled to 2 °C/min by controlling the voltage supplied to nichrome winding.

RTT fibres have been treated with catechin at a concentration of 0.01 M (adjusted to different pH's viz., 4, 6, 8 and 10 using buffer) for 24 h at room temperature (27 °C) without any agitation. The pH of the solution has been determined using digital pH meter (PH 5652) of Electronics Corporation of India. Hydrothermal stability of treated fibres at different pH has been measured using micro-shrinkage tester as mentioned above.

2.3. Treatment with protein destabilizing reagent

Urea solutions of 1, 2, and 4 M have been prepared on a weight basis. The native and catechin treated collagen fibres have been equilibrated in urea solution for 24 h and then the hydrothermal stability of the fibres have been measured using micro-shrinkage tester.

2.4. Isolation and characterization of collagen solution

Acid soluble rat tail tendon (RTT) type I collagen is isolated according to the method described by Chandrakasan et al. [20]. The procedure included acetic acid extraction and salting out with NaCl. The purity of collagen preparation has been confirmed by SDS-polyacrlyamide gel electrophoresis. The collagen concentration in the solutions has been determined from the hydroxyproline content according to the method of Woessner [21].

2.5. Investigation on conformational changes

Circular dichroic spectrum of native collagen in 5 mM acetic acid has been recorded in the far UV region using Jasco 715 Circular Dichroism spectropolarimeter. The collagen solution has been treated with different concentrations of catechin (0–240 μM) and the reaction mixture has been investigated for any conformational changes in the native collagen structure.

2.6. Interaction studies with collagen film

The collagen solution prepared from RTT [18] has been cast on a glass and air-dried in a laminar flow hood. The collagen film has been treated with 0.01 M catechin for 24 h at room temperature. The treated collagen films are washed with water, air-dried and their FT-IR spectra recorded using a Perkin-Elmer RX I FT-IR spectrophotometer.

2.7. Computational details

Catechin molecule has been built using builder tools outfitted with Silicon Graphics O2 workstation. Consistent Valence Force Field (CVFF) has been assigned to all atoms of the polyphenol molecule. The geometry of the catechin has been minimized using steepest decent method followed by conjugate gradient algorithm. Energy minimized coordinates of catechin have been used to estimate its size and surface areas using Connolly method as implemented in Insight II software package.

2.7.1. Interaction of polyphenols with collagen triple helix

The molecular simulation of huge collagen molecule is feasible by restricting the number of repeating units. In the present study 24-mer collagen triple helix is constructed by Object Technology Framework (OTF) using the GENCOLLAGEN package [22]. The 24 residue long triple helix constructed corresponds to the residues 193–216 ($2\alpha_1$ and $1\alpha_2$ chains) of the native type I collagen except residue 204 of α_1 chain. Residue 204 of α_1 chain, alanine is replaced by lysine in order to study the interaction of polyphenolic molecules with the side chains of basic amino acids.

Following is the amino acid sequence of α_1 chain of the triple helix, which is represented by standard three letter codes of amino acids: