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Effect of D-amino acids on collagen fibrillar assembly and stability: Experimental and modelling studies

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ABSTRACT

The effect of selected D-amino acids (D-AAs) on collagen with 1-ethyl-3-(3-dimethylamino propyl)carbodiimide (EDC)/N-hydroxysuccinimide (NHS) initiated crosslinking is evaluated by using experimental and modelling tools. The experimental results suggest that D-Lysine (D-Lys) plays a pivotal role in the self-assembly and conformation of collagen fibrils than D-Alanine (D-Ala) and D-Glutamic acid (D-Glu). The SDS-PAGE, absorption spectrum and viscosity measurements indicate significant differences in the D-Lys crosslinked collagen when compared to other systems. The CD spectra show an increase in the peak intensity at 220 nm in the presence of D-Lys, which could be due to increase in propensity of the structure to form a triple helix. Modelling studies indicated that D-Lys bind with collagen-like peptide (CLP) through multiple H-bonding and hydrophobic interactions. D-Lys has the lowest binding energy (−4.2 kcal/mol, indicating strongest interactions) when compared to D-Ala and D-Glu (−3.6 and −3.7 kcal/mol, respectively). Orientational changes in the collagenase on CLP-D-Lys are observed which may decrease its accessibility to degradation and stabilise CLP against the action of the former. D-Lys has the lowest binding energy and improved fibrillar-assembly and staggered alignment without the undesired structural stiffness and aggregations. The information derived from the present study could help in designing heterochiral collagen-based biomaterial.

1. Introduction

The molecular mechanisms of collagen fibrillar assembly and stability are of great significance for both basic research and preparation of collagen based biomaterials [1,2]. The ability of collagen molecules to assemble into crosslinked fibrils is an important requirement for the development of extracellular matrix (ECM). Covalent, non-covalent intermolecular, intra molecular crosslinking, electrostatic interactions (salt bridges), hydrophobic interactions and H-bonds have been the contributing factors for the self-assembly and stabilisation of collagen. The extent of interactions exhibited by functional groups of collagen with the crosslinker affect the interlayer spacing of the collagen fibril [3–5]. The chemical crosslinking involves the formation of either intra or intermolecular ionic or covalent bonds between the amino acid (AA) residues of collagen. These physical crosslinking methods viz. photooxidation, dry heat and exposure to UV or γ-irradiation are shown to be convenient since they do not require any chemical additives. Various physical crosslinking treatments themselves already induce amide formation and esterification between —COOH, $—NH_2$ and —OH groups, but the effect is insignificant during typical treatment conditions. However, collagen matrix becomes partially denatured, thereby yields an insufficient degree of crosslinking [6]. Other chemical crosslinking methods using inorganic polyvalent cations, including trivalent chromium, aluminium, etc., and organic aldehydes, carbodiimides, polyepoxy compounds, etc., have been used successfully. However, these methods significantly interfere with the mobility, strength and biocompatibility of the biomaterial [6].

1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC)/N-hydroxysuccinimide(NHS) crosslinking system has been frequently used in the treatment of collagen, which has shown better biocompatibility than aldehydes [7]. The crosslinking takes place due to the reaction between the carboxyl groups of glutamic/aspartic acid residues and the amine groups of lysine of collagen, thereby forming amide bonds. The crosslinking method using EDC/NHS induces the formation of an amide bond by the activation of the side chain —COOH group of aspartic and glutamic acid, followed by aminolysis of the O-iso acylurea intermediates by the

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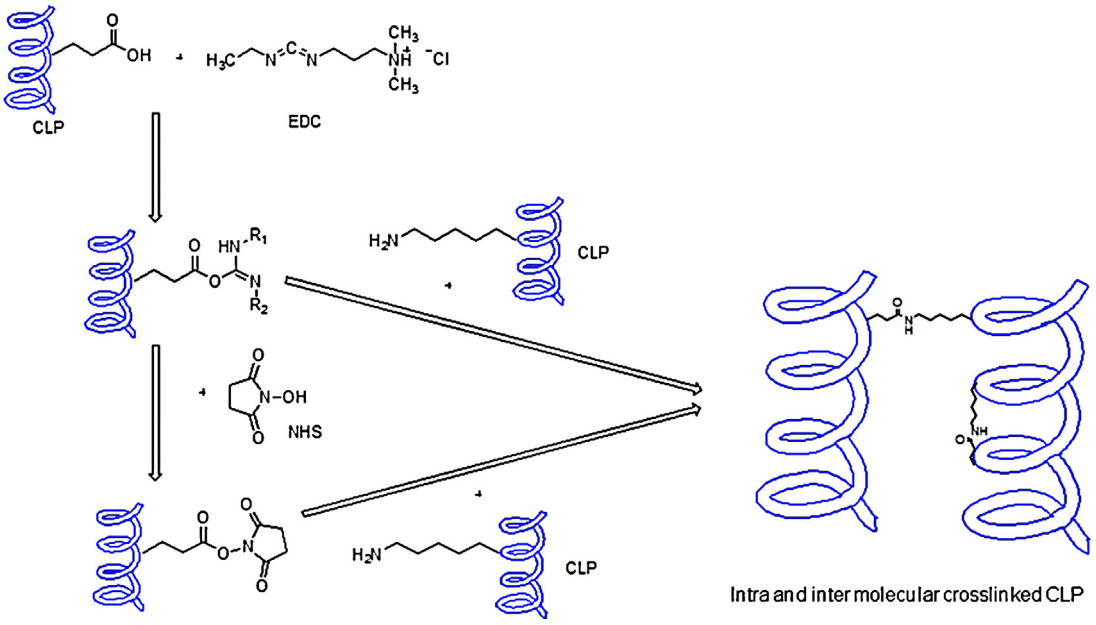

Scheme 1. Schematic representation of EDC/NHS initiated crosslinking bridge between collagen molecules.

$-NH_2$ groups of (hydroxy)lysine. This leads to the formation of intra- and inter-helical crosslinks (Scheme 1). There are also some drawbacks in using EDC/NHS as a crosslinking agent. It is a zero length crosslinker which produces collagen matrices with increased stability but it also leads to undesired stiffening of the collagen fibrils. This stiffness may result in the deterioration of its function and even in long-term failure of the collagen-based implant.

EDC/NHS and glutaraldehyde methods have been used to crosslink the collagen and leather matrices respectively, in the presence of D-amino acids (D-AAs), which serve as the crosslinking bridges [8–11]. Recent computational study has suggested that the replacement of AAs residues from L- to D-conformation stabilise the collagen triple helix. These D-AAs proteins and peptides have longer-acting anti-microbial, antioxidant, anti-mutagenic and anti-carcinogenic activities than their L-enantiomeric analogues [12]. The D-AAs containing peptide and protein fragments are less digestible than the L-enantiomers. They are the most effective chemopreventive agents against the initiation, promotion and progression stages in multistage carcinogenesis [12]. These peptides have contributed to innate host defense against a number of microbial pathogens. D-AAs endow resistance to proteolytic degradation, an attribute important in chemotherapeutic application [13]. Replacement of glycine residues with D-AAs in several globular proteins has greatly enhanced the conformational stability [14]. Applications of molecular modelling for the design of orthopaedic, dental and cardiovascular biomaterials have been studied [15]. The experimental and modelling study of collagen scaffolds and the effects of crosslinking and fibre alignment have been reported [16]. The aromatic interactions promoting the self-assembly of collagen triple-helical peptides to higher-order structures have been analysed [17]. Therefore, an understanding of the role of D-AAs on collagen self-assembly can help in designing novel matrices. The screening of potential and novel polymeric biomaterial candidates by computational method plays an essential role in drug delivery and tissue engineering.

The present study is to understand the self-assembly and stabilisation of collagen with EDC/NHS in the absence and presence of D-AAs using experimental and theoretical studies. The interaction of three D-AAs with their energy minimised triple helical structures of collagen-like peptide (CLP) was studied using molecular docking techniques in order to determine the stabilisation of the latter. The orientational changes in the CLP binding domain in *Clostridium histolyticum* class I collagenase (ChC) due to the crosslinking of AAs were also studied. Furthermore, the self-assembly and modelling results were compared with the experimentally determined mechanical strength, hydrothermal stability, resistance to biodegradation and biocompatibility of collagen–D-AAs scaffolds. SDS-PAGE, CD spectral, viscosity measurements of the systems were carried out to rationalise the theoretical observations.

2. Experimental procedures

All reagents and chemicals used were analytical grade. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxy succinimide (NHS) were sourced from Fluka and Sigma Chemicals Co., USA. All other reagents and chemicals used for the study were sourced from SRL Ltd., India.

The computational calculations comprise of determining the energy minimised three-dimensional structures of CLP and D-AAs by using Autodock Software (Autodock 4.2) [18]. The possible orientational changes of the active site of ChC on CLP-D-AAs are determined by protein–protein docking procedure (PatchDock) [19–22]. The crystal structure of the triple helical CLP for molecular docking studies has been obtained from the Protein Data Bank (PDB: 3A1H) (Fig. 1A). The —OH, —COOH and $-NH_2$ in the AAs have been considered as potential interacting sites for the formation of H-bonds with D-AAs. AAs having similar functional groups have not been repeated in the model peptide sequence. The single-crystal structures of the three collagen-like host–guest peptides are (Pro-Pro-Gly)(4)-Hyp-Yaa-Gly-(Pro-Pro-Gly)(4) [Yaa = Thr, Val, Ser; Hyp(4R)] [28]. For the current study, the structures of the three D-AAs namely, D-Alanine (CID: 71080), D-Glutamic acid (CID: 23327) and D-Lysine (CID: 57449) were retrieved from Pubchem Compound Database. The crystal structure of a collagen-binding domain (CBD) with a N-terminal domain linker from *C. histolyticum* class I collagenase (ChC) determined at a resolution of 1.00 Å in the absence of calcium (1NQJ) and at a resolution of 1.65 Å in the presence of calcium (1NQD) is chosen for this study (Fig. 1B).