Materials Science and Engineering C 57 (2015) 249–256

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Does L to D-amino acid substitution trigger helix → sheet conformations in collagen like peptides adsorbed to surfaces?

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ARTICLE INFO

Article history:
Received 1 September 2014
Received in revised form 22 June 2015
Accepted 28 July 2015
Available online 1 August 2015

Keywords:
Collagen like peptide
Chirality
Adsorption
D-amino acid
Dichroism

ABSTRACT

The present work reports on the structural order, self assembling behaviour and the role in adsorption to hydrophilic or hydrophobic solid surfaces of modified sequence from the triple helical peptide model of the collagenase cleavage site in type I collagen (Uniprot accession number P02452 residues from 935 to 970) using ^{D}Ala and ^{D}Ile substitutions as given in the models below:
Model-1: GSOGADGPAGAOGTOGPQGIAGQRGVV GLOGQRGER.
Model-2: GSOGADGP**^{D}A**GAOGTOGPQGIAGQRGVVGLOGQRGER.
Model-3: GSOGADGPAGAOGTOGPQG**^{D}I**AGQRGVVGLOGQRGER.
Collagenase is an important enzyme that plays an important role in degrading collagen in wound healing, cancer metastasis and even in embryonic development. However, the mechanism by which this degradation occurs is not completely understood. Our results show that adsorption of the peptides to the solid surfaces, specifically hydrophobic triggers a helix to beta transition with order increasing in peptide models 2 and 3. This restricts the collagenolytic behaviour of collagenase and may find application in design of peptides and peptidomimetics for enzyme–substrate interaction, specifically with reference to collagen and other extra cellular matrix proteins.

1. Introduction

Knowledge about protein adsorption to solid surfaces in an aqueous environment is crucial in a number of applications in biomaterials. For example in biomedicine, it is important to understand the adsorption of blood proteins to implants. In some cases protein adsorption needs to be minimized, for instance by fouling of heat exchangers or for adsorption to ultra filtration membranes. It's well known that protein adsorption to surfaces is influenced by numerous factors such as peptide/protein sequence, conformation, and solvent conditions [1–5]. Tsai et al. have shown improved adsorption of antimicrobial indolicidin-derived peptides to hydrophobic surfaces compared to hydrophilic substrates [6]. Somorjai and co-workers have studied adsorption of aqueous amino acids, homo-dipeptides, and hetero-dipeptides of phenylalanine, lysine, and glycine and proteins to hydrophilic and hydrophobic surfaces using sum frequency generation vibrational spectroscopy (SFG) and quartz crystal microbalance (QCM) [7–9]. Results show that the phenyl ring in phenylalanine adsorbs nearly flat with respect to the hydrophobic surface, when not constrained by other adsorbates [10].

Inserting D-amino acid residues in proteins and peptides in an L-amino acid environment is known to affect the three dimensional structure of proteins and peptides, which can in turn affect the adsorption [11–15]. The destabilization resulting from L → D-amino acid substitution can be used to probe the structure activity relationship between conformational domains and bioactivity [16]. ^{L}Ala → ^{D}Ala substitution destabilizes the right handed α-helical secondary structure by 0.96 kcal/mol and adjacent pair of D-amino acid substitution leads to destabilization of ^{D}Ala and ^{D}Leu by 1.05 and 0.66 kcal/mol compared to that of L-enantiomers [13–17]. Rai et al., designed the β-hairpin peptide (Boc-Leu-Val-^{D}Pro-^{L}Pro-^{D}Ala-Leu-Phe-Val-OMe) using D-amino acids showed that ^{D}Pro acts as a conformational determinant and helps in forming artificial reverse turns while ^{D}Ala nucleates the formation of β-hairpin [18].

D-amino acids are found in proteins through posttranslational modification reactions and racemization. Slow and spontaneous racemization is found in proteins for example, in cataract formation, crystallin, and collagen [19,20]. Spontaneous racemization in collagen is well known and successfully used in dating of biological specimens [21]. Variation in the rate of racemization of L-amino acids in protein is dependent on different residues, temperature and pH [22–24]. In collagen, the rate of racemization of different amino acids is as follows: Asp > Ala > Glu ~ Leu > Ile [25]. Collagen is a unique protein with a triple helical structure. It consists of three polypeptide chains, which form extended left handed polyproline II like conformation, that intertwine parallel with each other by staggering of one residue of each chain and

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http://dx.doi.org/10.1016/j.msec.2015.07.057
0928-4931/

forming a right handed triple helix. The left handed α-chains have unique characteristic of Y_{AA}-X_{AA}-Gly triplets repeating unit, where X_{AA} and Y_{AA} can be any L-amino acid, most frequently Lproline and Lhydroxyproline [26–28]. Each α-chain includes both polar and apolar residues in primary amino acid sequence and are grouped in alternative manner as hydrophilic and hydrophobic patches.

Model peptides with the X_{AA}-Y_{AA}-Gly repeats have been widely used in the past to understand collagen folding, packing and in structural elucidation of unique triple helical conformation. Till date fibril forming collagen mimetic peptides have exploited hydroxyproline based, thermally stabilized sequences [29–44]. Collagen like peptides provide an insight into the engineering of novel collagen like biomaterials. Most commonly, Pro-Hyp-Gly triplets found to an extent of 10% in collagen have been used because this triplet forms a stable triple helix [45–53]. Effect of single substitution of Gly with LAla, DAla, β-Ala and sarcosine at the C and N-terminal was analyzed by Chen et al., to explain the position dependant destabilization [15]. The effect of D-amino acid substitution in Gly position in collagen like peptides has been addressed by Raines and co-workers. They have suggested that, replacement of Gly residue with DAla and DSer residues also leads to the formation of triple helical conformation [54]. Shah et al., found through thermal analysis that D-Asp substitution in collagen like peptide sequence prevents triple helical formation. But it favours the formation of hetero-trimer triple-helical molecules under mixture of DAsp and LAsp [19]. In our earlier studies, host-guest peptide approach combined with MD simulation had been utilized to explore the effect of D-amino acids (Asp, Ala and Pro) on the structure and stability of collagen. Results reveal that, D-AA substitution in the collagen like peptides tends to form kink at the substituted site. Interestingly, D-AA substitution does not only not unwind the triple helix, but also results in more stable DAla substituted triple-helical model compared with that of DPro and DAsp [11].

In contrast to the extensive investigation on Pro-Hyp-Gly repeats (imino rich region), studies on the natural sequences have been seldom reported since natural sequences usually fail to form a stable triple helix under normal conditions. Self-Assembly of collagen-mimetic peptide amphiphiles into bio-functional nanofiber has been reported by Luo and Tang [55]. Higher-order assembly of collagen peptides into nano- and micro-scale materials have been reported by Przybyla and Chmielewski [56]. The role of collagen in the aging process of fossil materials has been evaluated by a simple and accurate method by determining the extent of deamidation at the whole protein level [57]. In earlier studies, imino poor regions substituted as a guest in Pro-Hyp-Gly triplet repeats (used as host) showed destabilization [58,59]. In collagen sequence it has been noticed that Pro-Hyp-Gly consecutive triplet repeats are scarce. In contrast to the numerous studies carried out on the stability of the collagen like peptide with Pro-Hyp-Gly repeats with L-amino acids, little is known about the effect of D-amino acids in these peptides and native imino poor regions.

In the present work, the natural sequence from the triple helical peptide model of the collagenase cleavage site in type I collagen (Uniprot accession number P02452 residues from 935 to 970) has been constructed using DAla and DIle substitutions. This L to D conversion fails to form stable triple helix as well as restricts the collagenolytic behaviour of collagenase [60]. It is expected that construction of natural sequence which mimics the properties of native collagens will be helpful in understanding self-assembling behaviour and also in developing collagen mimetic materials. In this study, we report on the effect of DAla and DIle substitution in native sequence of collagen mimics and their organization on adsorption to hydrophilic and hydrophobic solid surfaces.

2. Experimental section

2.1. Peptide design

Three peptides were custom synthesized by the advanced protein chemistry laboratory, Tufts University, USA using standard solid-phase Fmoc chemistry. The synthesized peptide sequences designs are based on imino poor regions of α_1 chain of type I collagen in PDB number P02452 residues from 935 to 970 including Hyp at Y_{AA} position with D-amino acid substitutions. All other chemicals and solvents used were analytical grade. Amino acids are represented in the single letter codes and hydroxyproline residue is represented as O. The terminals are blocked with acetyl (Ac) and N-methyl (Nme) groups. Amino acid sequence and its structure have been shown in Fig. 1. Model 1 is a native imino poor sequence of α_1 chain of type I collagen in PDB number P02452 residues from 935 to 970 including Hyp at Y_{AA} position. In peptide models 2 and 3, DAla and DIle, respectively, replaced LAla and LIle in the third and seventh triplets. In addition, these sequences have GER motif, which is an essential triplet to enhance the cell adhesion activity and these charged motifs are thermally stable in the absence of Hyp.

2.2. Preparation of hydrophilic and hydrophobic surface

Quartz slides (Erma, FRG) were cleaned using chromic acid followed by repeated washing with Millipore water (Milli-Q, USA) and stored in dessicator. The surfaces were further cleaned using Plasma Cleaner (Harrick Plasma, USA) for 10 min, to ensure complete surface hydrophilicity. A slide from each batch was tested by measuring contact angle of water, which was about 7°. For preparation of hydrophobic surface, the quartz surface was made hydrophobic using the Langmuir–Blodgett technique (LB). The solution of stearic acid in chloroform was spread at the air/water interface and the solvent was allowed to evaporate for a period of 20 min. The quartz was withdrawn from the sub-phase, which was maintained at a constant surface pressure $\pi = 20$ mN/m whereby 1 layer of stearic acid LB film was transferred. The transfer ratio was 0.85.

2.3. Sample preparation

Aqueous solutions of the peptides were prepared with the concentration of 1 $mg.mL^{-1}$. The solutions were heated at 60 °C for 5 min then immediately stored at 4 °C for a minimum period of 72 h to allow formation of triple helical conformation. 50 μL peptide solutions ($1\ mg \cdot mL^{-1}$) were adsorbed onto hydrophilic and hydrophobic surfaces of the quartz slides to form a thin layer of peptide on the surfaces and slides were then allowed to dry at room temperature in a vacuum desiccator.

2.4. Circular dichroism (CD) measurements

The sample slides were subjected to Far-UV CD measurements using Jasco J-815 CD spectrophotometer model equipped with a Peltier temperature controller-423S/15 (JASCO Inc.). CD spectra of the sample slides were scanned between 260 to 190 nm at 25 °C using 1.0 nm band width, 0.1 nm step size, for an average time of 1 s with an average of three scans at a scan speed of 100 nm/min. Data analysis was performed with CONTIN software packages [61–63].

2.5. Fourier Transform Infrared Spectroscopy (FT-IR)

Peptide solution ($0.5\ mg^{\circ}mL^{-1}$) was adsorbed onto low-e glass microscope slides and kept for drying in a vacuum desiccator. The dried film was investigated by reflectance infrared spectroscopy (ABB MB 3000 Instruments, Pike Technologies, USA) using an average of 80 scans at a resolution of 4 cm^{-1}.

2.6. Interferometry measurements

To evaluate change in thickness on adsorption of the peptides on hydrophilic and hydrophobic surfaces, thickness was measured using Filmetrics F20 UV thin film analyzer. All values are average of three measurements on the surface and with a minimum goodness of fit 0.97.