Carbohydrate Research 389 (2014) 12–17

Contents lists available at ScienceDirect

Carbohydrate Research

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

Carbohydrate-functionalized collagen matrices: design and characterization of a novel neoglycosylated biomaterial

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ARTICLE INFO

Article history:
Received 4 September 2013
Received in revised form 21 October 2013
Accepted 11 November 2013
Available online 17 November 2013

Keywords:
Neoglycosylation
Reductive amination
Lactose
Collagen
AFM
Lectins

ABSTRACT

Collagen matrices have been neoglycosylated with lactose by reductive amination at lysine side chains. AFM analysis highlights that the chemical does not affect molecular assembly into fibrils. Moreover, ELLA biochemical assays show that the glycan moiety is efficiently exposed on the matrix surface for receptor recognition.

1. Introduction

The design of new protein-based biomaterials has mushroomed over the past decade. Works in this field present both challenges in basic science such as understanding of biomolecular assembly mechanisms, and opportunities in material science, bionanotechnology and for applications in tissue engineering. At present there is a pressing need to selectively modify peptides and protein matrices to produce functional biomaterials for tissue engineering applications.[1] These biomaterials can be very challenging to prepare due to the high level of functionality and of structural complexity of the protein themselves. Because of the higher accessibility surface, linear or fibrous proteins offer a prised solution with respect to globular proteins: the outer exposition of the amino acid side chains observed in the first class of protein is unviable for the other class. One of the main representatives of the first family is collagen. Collagen molecule has a triple-helix structure[2] formed by the assembly of three polypeptide chains each in a polyproline-II like conformation and supercoiled around a common axis. The three chains are staggered by one residue and their close packing near this axis requires every third amino acid to be glycine (G) thus generating the characteristic G-X-Y repeating pattern observed in collagen. Among collagens, collagen type I represents the main molecular building block of all the connective tissues involved in structural functions. Both its primary sequence (NP_000079, a1(I); NP_000080, a2(I)) and its conformation are known thus allowing to assess the position of a given residue type along the molecule and hence to identify the position and the accessibility of feasible binding sites along the collagen molecule. In the case of type I collagen the triple-helix structure is stabilized by its high content of Proline (P) and hydroxyproline (O) which promote PPII helix stability. The triple helix structure of collagen allows short peptide sequences to be used for the study of collagen stability both experimentally and theoretically. Accordingly the high propensity observed for the triplets G-P-O reflected on the sequences studied in the literature so far.

In recent years, the development of collagen based functional materials has gained a lot of interest.[3] Such materials are useful as scaffolds for cell growth, for wound healing and the development of artificial skin. The design of collagen matrices that can be easily functionalized with signalling biomolecules in order to upgrade collagen to a cell-responsive biomaterial, without altering its structural features is of primary importance in order to preserve its native recognition and biomolecular properties. Despite their

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http://dx.doi.org/10.1016/j.carres.2013.11.008

noted functional role,[4] glycans have had limited use as signalling epitopes for biomaterial surface decoration.[5] Furthermore, in recent years, collagen glycosylation is emerging as a key issue in the control of cartilage formation, growth, metabolism and repair.[6]

In the present work we explored whether collagen-based 2D matrices may be functionalized with glycan moieties without affecting their structural features. In this respect, theoretical and molecular modelling are valuable tools to guide the design of collagen-based materials.[7] Performing residue-specific chemical reactions on collagen and maintaining its integrity is not an easy task.[8] Different strategies have been proposed during the years for protein bioconjugation;[9] most of them usually target functionalities present in the side chains of the canonical, proteogenic amino acids, where cysteine[10] and lysine[11] are the most exploited residues.

In the proposed work, a bioconjugation approach targeting lysine residues was chosen for the linkage. Lysines bear a primary amine moiety reactions that have proven useful for bioconjugation is the coupling of the amino group with carbonyls, affording the corresponding Schiff base or a secondary amine after imine reduction.[12] Collagen is reported to be usually glycosylated with small glycidic epitopes, β-galactosides or α-(1-2)-glucosyl-β-galactosides.[13] Hence, lactose was chosen as a model carbohydrate, since upon reductive amination it exposes β-galactosides, and moreover, because the main aim of the work is to assess by theoretical and experimental methods if a widely used procedure for neoglycosylation targeting lysine side chains (i.e., reductive amination) may have detrimental consequences on collagen structure, that is known to be fundamental for the exploitation of its functions.

2. Result and discussion

2.1. Molecular modelling of lysine-specific modification of collagen

We chose the simplest model of collagen, with only Glycine–Proline–Hydroxyproline (GPO) triplets on each of the three chains in order to represent an archetype of collagen molecule. The collagen model we use, $[(GPO)_5]_3$, is truncated to 15 amino acids per chain due to computational limitations, since the full length collagen molecule (300 nm long) is too large for atomistic simulations. This leads to a collagen-like segment with a length of approximately 7 nm. Peptides of comparable length have been used both in earlier computational and experimental studies.[2b,14] In one of the three chains, the proline residue in the central triplet is substitute by lysine (the functionalization point). In addition to the wild type case, the functionalization with lactose has been tested, where the disaccharide is linked to the side-chain of lysine (Fig. 1).

Molecular dynamics simulations are performed to assess the equilibrium configuration of the wild-type and functionalized collagen molecule (Fig. 2). The results show that the wild-type lysine is exposed towards the solvent although it lies close to the triple helix. On the other hand, the lactose functionalization is well exposed and particularly the glucose ring is highly solvated. Partial charge calculations show that lysine side chain presents neutral or mildly positive partial charges, except for the protonated amine group, which has high partial charges. Conversely, the lactose functionalization, bearing several –OH groups, shows a high number of charge dipoles which greatly contribute to its hydrophilicity.

2.2. Neoglycosylation of collagen

Collagen Type I from equine tendon was used for the preparation of 2D matrices by solvent casting method.[15] Neoglycosylation of collagen (Scheme 1) was achieved reacting lysine side-chain amino groups with 0.06 M lactose aq solution in 0.03 M $NaCNBH_3$ in citrate buffer (pH 6.00).

2.3. Morphological characterization

The untreated collagen film observed by Tapping-Mode Atomic Force Microscopy reveals a moderately rough, grainy surface interspersed by long, straight collagen fibrils of various size and

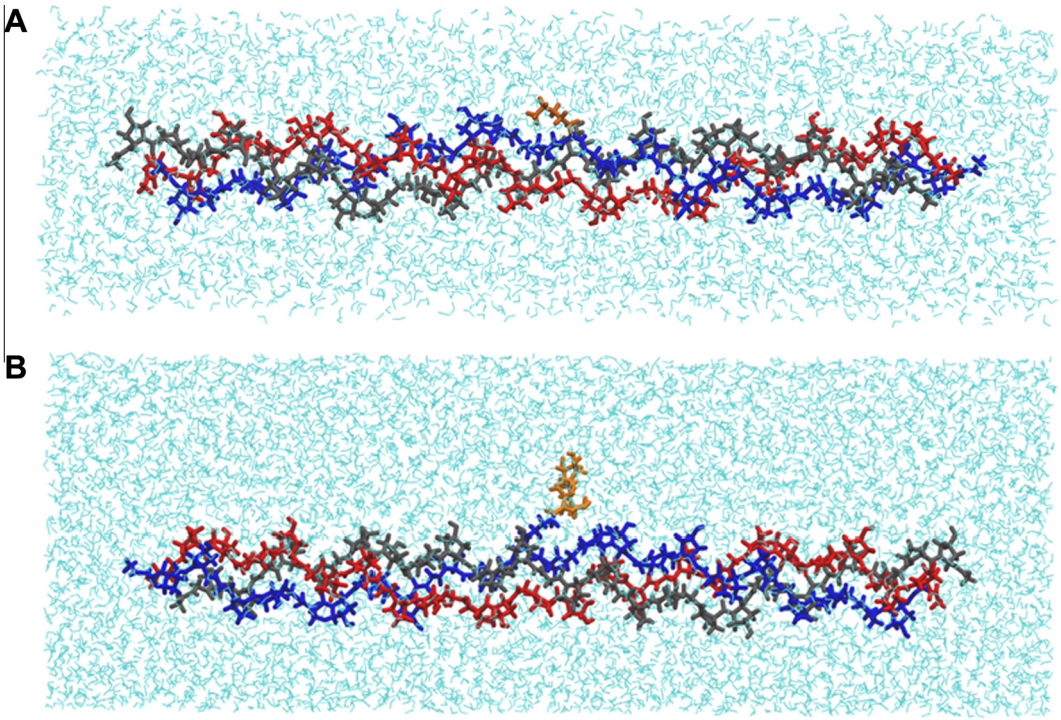

Figure 1. Initial structures used for MD simulations. The wild type peptide features GPO triplets except for a central lysine, shown in orange (Panel A). In the functionalized collage lactose is covalently attached to the side-chain of lysine (orange in Panel B). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)