MOLECULAR ANALISYS OF INTERACTION ENERGIES OF THE DECORIN PROTEOGLYCAN - COLLAGEN COMPLEX IN TENDON FIBRILS

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INTRODUCTION

It is well known that tendon mechanical functions are related to tissue structural components and their organisation. Nevertheless the molecular and sopramolecular interactions in tendons have not been exhaustively studied yet. In tendon fiber, collagen type I fibrils are found associated with small proteoglycans (PGs) (1). The majority is represented by decorin, a small PG composed by a globular protein core and a single glycosaminoglycan (GAG) chain (2).

Collagen I molecules have a characteristic triple-helical conformation due to the repetition of triplets Gly-X-Y (Gly, X and Y stand for glycine and two general residue, respectively).

Decorin core protein is globular and arch shaped (3), its concave surface links non-covalently to the collagen molecule at the fibril gap regions every 68 nm (1).

Different GAG chains can be detected mainly composed by chondroitin sulfate, dermatan sulfate or keratan sulfate. Recent studies have shown that GAGs bounded to decorin act like bridges between contiguous fibrils connecting adjacent fibril and they are mainly aligned orthogonally to the major axis of collagen fibril. This architecture would suggest their possible role in providing the mechanical integrity of the tendon structure (4).

Many works state that decorin contains high affinity binding site for collagen type I (3). The main decorin binding site for collagen is known like the GAG attachment site (3), on the contrary collagen main binding site for decorin is still an open question (3). The objective of the study is to define topological and mechanical features of these three components by means of the molecular mechanics methods (AMBER 3) (5).

Collagen like triple-helix peptides alfa1$(I)_3$ have been modelled using the resides sequence 949-979 which seems to be a good candidate to behave like the main collagen binding domain (3).

Three-dimensional structure of human decorin has been obtained starting from decorin tertiary structure previously studied (3) based on the crystal structure of the porcine ribonuclease inhibitor (PBD ID 1DFJ).

With reference to GAG chain, an atomic model of nine units of three GAG chains (chondroitin-sulfate, dermatan-sulfate and keratan-sulfate) have been developed with the aim to investigate the ability of GAGs in transmitting the force between adjacent fibrils.
Affinity level between decorin and collagen triple-helix is obtained by means interaction energy evaluation. GAG deformation energy are calculated from energy data obtained by progressively stretching the molecular structure.

MATERIALS AND METHODS

Collagen like molecule

Collagen like molecule is composed by three identical helices alfa1(I) each composed by 30 residues; collagen binding domain centres at residues 961-962. Collagen tertiary structure (the triple-helix) has been obtained starting from the collagen like peptide structure T3-785 (PBD ID 1BKV). Each alfa1-helix was modelled and subsequently they were superimposed. The diameter rod formed by the collagen like molecule is around 1.5 nm. This structure has been optimised using the molecular mechanics AMBER 3 force field, in order to obtain the superhelical structure arrangement of the alfa1$(I)_3$ assembly. Figure 1 shows the optimised collagen like molecule.

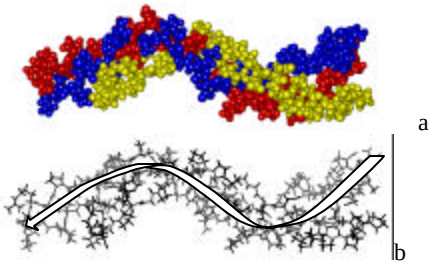

Figure 1. Superhelical structure of the collagen like molecule, each alfa-helix is depicted by different colour a), right handed arrangement is shown b)

Decorin core protein

Decorin structure is arch shaped with an inner concave surface formed by the curved beta-sheets and an outer convex surface formed by alfa-helices (Fig.2). The distance between the two arms is about 6.5 nm whereas the distance between the base of the arch and the peak is 4.5 nm and the overall thickness is equal to 3 nm.

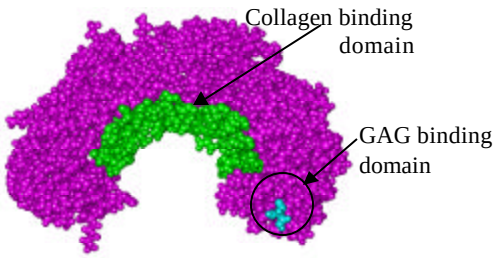

Figure 2. Decorin core protein. Collagen binding domain (green) and GAG binding domain (cyan)

Glycosaminoglycan chain

Three different GAG chains of chondroitin sulfate (CS), dermatan sulfate (DS) and keratan sulfate (KS), each composed by nine disaccharide units have been modelled (Fig.3, left). Starting from the optimised GAG chain calculated at resting conditions in a wavy arrangement, sequential stretches have been imposed and full optimisation has been performed in order to attain the minimum energy configuration (Fig.3, right). GAG deformation energy are calculated from energy data obtained by progressively stretching the molecular structure.

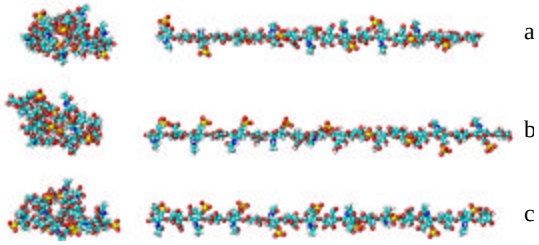

Figure 3. Molecular models of CS a), DS b), KS c)

RESULTS

Collagen molecule-decorin core protein interaction energy

Geometry optimization is performed to evaluate intramolecular interaction energy, during optimization only the binding regions of these two structures are free to move (decorin in green and collagen in blue, Figure 4), this solution reduces calculation time. Given the optimized energy of these complex E_{tot}, being E_{dec} and E_{col} the energy values of decorin and collagen molecule respectively, interaction energy (E_{int}) is given by equation $E_{int}=E_{tot}-E_{dec}-E_{col}$. With respect to this configuration interaction energy is equal to 546.75 kcal/mol and it has an attractive feature.

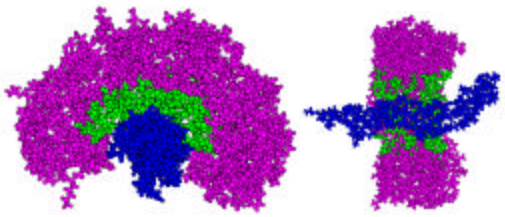

Figure 4. Decorin - collagen complex

Glycosaminoglycan stretching energy

Each GAG chain shows a similar shape in the Potential energy/strain function (Fig.5), whereas each GAG shows different limit values (Tab.1).

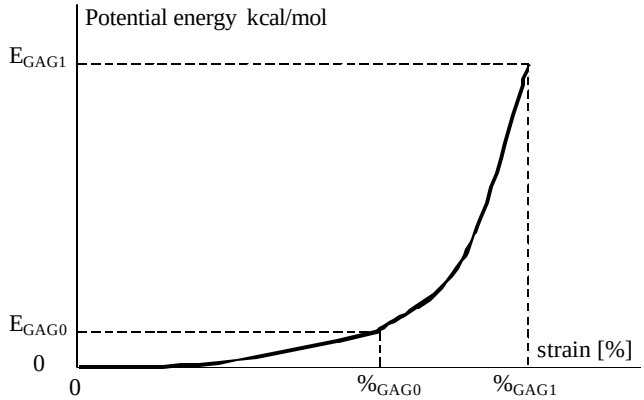

Figure 5. GAG potential energy as a function of GAG strain

GAG	$E_{GAG0\ [kcal/mol]}$	$E_{GAG1[kcal/mol]}$	$\%_{GAG0[\%]}$	$\%_{GAG1[\%]}$
CS	75.68	2170.64	839	1093
DS	46.39	1538.73	960	1361
KS	93.57	2050.78	773	985

Table1. Deformation energy and strain limit values for different GAG chains

DISCUSSION

This study confirms that decorin core protein and collagen molecule show an attractive intramolecular energy, moreover by means of molecular mechanics approach this interaction can be confronted with GAG deformation energy. In particular in the beginning, GAG deformation energy seems the main actor, secondly further stains give rise to GAG deformation energy bigger than decorin-collagen interaction energy, this zone needs additional analysis. The idea is that decorin shifts along collagen molecule in order to decrease GAG energy.

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