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Estimation of the binding force of the collagen molecule-decorin core protein complex in collagen fibril

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Abstract

Decorin belongs to the small leucine proteoglycans family and is considered to play an important role in extracellular matrix organization. Experimental studies suggest that decorin is required for the assembly of collagen fibrils, as well as for the development of proper tissue mechanical properties. In tendons, decorins tie adjoining collagen fibrils together and probably guarantee the mechanical coupling of fibrils. The decorin molecule consists of one core protein and one glycosaminoglycan chain covalently linked to a serine residue of the core protein. Several studies have indicated that each core protein binds to the surface of collagen fibrils every 67 nm, by interacting non-covalently to one collagen molecule of the fibril surface, while the decorin glycosaminoglycans extend from the core protein to connect to another decorin core protein laying on adjacent fibril surface. The present paper investigates the complex composed of one decorin core protein and one collagen molecule in order to obtain their binding force. For this purpose, molecular models of collagen molecules type I and decorin core protein were developed and their interaction energies were evaluated by means of the molecular mechanics approach. Results show that the complex is characterized by a maximum binding force of about 12.4×10^3 nN and a binding stiffness of 8.33×10^{-8} N/nm; the attained binding force is greater than the glycosaminoglycan chain's ultimate strength, thus indicating that overloads are likely to damage the collagen fibre's mechanical integrity by disrupting the glycosaminoglycan chains rather than by causing decorin core protein detachment from the collagen fibril.

Keywords: Decorin; Collagen molecule type I; Collagen fibril

1. Introduction

From a structural standpoint, tendons and ligaments can be considered fiber-reinforced composites made of relatively short collagen fibrils (prevalently type I) whose length is in the millimeter range (Trotter and Wofsy, 1989; Mosler et al., 1985; Folkhard et al., 1987; Parry and Craig, 1984; Craig et al., 1989; Kadler et al., 1996; Fratzl et al., 1997; McBride et al., 1988; Graham et al., 2000). Fibrils are embedded in a softer interfibrillar matrix mainly made of proteoglycans (PGs) bound to a collagen fibril surface (Scott 1988, 2001; Raspanti et al., 2002).

Collagen fibrils have a recurring structure due to the high-ordered assembly of collagen molecules. In particular, the most common collagen families—types I, II, III, V, and XI—form 67 nm periodic fibrils and this length is called the *D*-period. Since the length of one type I collagen molecule is 4.4*D*, the non-integral relationship between molecular length and the *D*-period length implies that an empty space remains between the end of each molecule and the beginning of the next one. According to the quarter stagger model (Petruska and Hodge, 1964), this pattern produces a periodic fibril structure whose basic unit, the microfibril, is composed of five molecules divided into overlap and gap zones (0.4 and 0.6*D*) where axial regions consist of five segments (overlap zones) and four segments (gap zones), respectively.

As shown by several authors, small leucine-rich PGs attach at the gap regions (Scott and Orford, 1981; Scott,

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1988, 1992; Hocking et al., 1998; Raspanti et al., 2000). In general, they are usually bound to collagen fibrils via their core protein and interact with each other via their glycosaminoglycan (GAG) side chains, as recently observed in different tissues (tendon, ligament, cartilage, skin, cornea) (Scott, 1992, 2001; Raspanti et al., 1997, 2000; Ottani et al., 2001, 2002). Decorin, the main PG in the tendon, belongs to this family of small extracellular matrix (ECM) proteoglycans and consists of one arch-shaped core protein covalently bound in the N-terminal region to a chondroitin or dermatan sulphate GAG chain (Scott and Orford, 1981; Chopra et al., 1985; Scott, 2001; Raspanti et al., 1997). Decorin plays an important role in the regulation of collagen fibrillogenesis, modulation of growth factor activity and preservation of ECM architecture. In fact, any disruption of the decorin gene is immediately reflected in the ECM architecture. Structure-function relationship in tendons was studied in transgenic mouse skin by Danielson et al. (1997). Quantitative analysis of individual collagen fibrils showed irregular outlines and size variability and uncontrolled lateral fusion of collagen fibrils in decorin deficient mice. The absence of these PGs was also studied by Elliot and co-workers in transgenic mouse tail tendons (Elliott et al., 2003). Again, the collagen network was loosely packed with irregular contours and fibrils fused together and mechanical testing ascribed tissue fragility to this abnormal matrix structure.

Another interesting feature of decorin core protein is that the binding region for the GAG chain is located at one edge of the decorin molecule (Weber et al., 1996). This feature gives mobility to the GAG, which can align orthogonally or parallel to the major axis of the collagen fibril following fibril rearrangement during loading, guaranteeing the mechanical coupling of fibrils and ultimately distributing the mechanical stress throughout the whole tissue. This ability was previously observed in an ultrastructural study by Scott (1988, 1992); in a tapping-mode atomic force microscopy study (Raspanti et al., 1997) and in a scanning electron microscopy analysis (Ottani et al., 2002). Recently, a model for this phenomenon was constructed by Redaelli et al. (2003). Through a multiscale approach, including molecular and classical mechanics models, this study investigated the functional contribution of the GAG chain to the overall mechanical performance of the tendon. Results showed that GAGs have an adequate stiffness to behave as stress transfer structures within tendon fiber. Fiber elastic modulus, compatible with GAG ultimate failure strain, would range from 77 to 475 MPa and is consistent both with data reported by McBride et al. (1988) concerning the developing tendon and with data on the mature tendon (Woo et al., 1981; Butler et al., 1984, 1986). Redaelli and co-authors' study, focused on GAG's mechanical elastic features and dismissed collagen molecule-decorin core protein interaction, which may constitute the weakest bond of the fibril-to-fibril system.

The specific association of decorin with collagen type I has been studied in recent years (Schönherr et al., 1995; Svensson et al., 1995; Keene et al., 2000; Tenni et al., 2002). Several studies indicated that each core protein binds non-covalently to an intraperiod site on the surface of collagen fibrils every 67 nm (Schönherr et al., 1995; Svensson et al., 1995; Scott, 2001), but the exact location is still debated and the binding force is unknown. The present work is aimed at filling in lacunae in the collagen fibril-decorin core protein-GAG chain complex, and it focuses on the evaluation of the interaction force between the collagen molecule and the decorin core protein. In greater detail, the present study was split into two tasks. The first was aimed at the definition and construction of the molecular actors, the collagen type I molecule and the decorin core protein; the second was aimed at evaluating the binding force between these complexes.

2. Materials and methods

The blueprint of the fibril-to-fibril assembly is shown in detail in Fig. 1. As shown by several authors, gaps, along collagen fibrils involve PGs attachment (Scott and Orford, 1981; Scott, 1988, 1992; Hocking et al., 1998; Raspanti et al., 2000). These regions, in agreement with the quarter stagger model (Petruska and Hodge, 1964), produce an empty space between collagen molecules where one arm of the arch-shaped decorin core protein would interleave so that the decorin concave surface would bind to a contiguous collagen molecule of the fibril. Considering two collagen fibrils, the assembly would involve two collagen molecules, each one part of a different fibril, two decorin core proteins and one GAG chain which interconnect the two fibrils. The following paragraphs describe the construction of the molecular models of the collagen molecule-decorin core protein assembly starting from the crystal structure of known peptides.

2.1. Collagen type I molecule modeling

The collagen molecule is characterized by a triple helix structure, and it is the structural element of the fibril forming collagen family (Brodsky and Shah, 1995). The nature of the triple-helical conformation was initially elucidated through X-ray fiber diffraction studies of native collagen (Ramachandran and Kartha, 1954) and, more recently, it was confirmed through X-ray crystallographic and computational studies of synthetic collagen-like peptides (Bella et al., 1994; Kramer et al., 1998, 2001). The triple helix of collagen