

Interactions of Proteoglycan and Collagen in Cartilage and Soft tissues

Keyword: Collagen – Proteoglycan – Glycosaminoglycans - Protein core - Stress - Strain.

Joel Sandé

Introduction

The purpose of this review is detailed criticism of articles content that focused on the factors involved in the interaction between collagen and proteoglycan in both "connective tissue" and "soft tissue". Judgments will be made on the quality of each article, comments on the findings of their methods and their common points.

The three papers submitted to our study talk about existing interactions between collagen fibers and proteoglycan; the first one « The interactions of cartilage proteoglycans with collagens are determined by their structures » is about the nature of interactions within cartilage, the second one « Mechanical interactions between collagen and proteoglycans: implications for the stability of lung tissue » is about the main actor of these interactions within lung tissue, and the third one « Structural Interactions between Collagen and Proteoglycans are elucidated by 3D Electron Tomography of Bovine Cornea » is about visualisation of these interactions in cartilage.

Interactions of cartilage proteoglycans with collagens are determined by their structure

Collagen and proteoglycans are the main macromolecules of cartilage. The first paper was undertaken to study the interactions of collagen and proteoglycans, and to detect the parts of their structures involved in such interactions.

The interaction of aggrecan, decorin and biglycan isolated from pig laryngeal cartilage and of the three squid cartilage proteoglycans with collagen type I and II was studied. The interaction was examined under conditions allowing formation of collagen fibrils.

The methodology applied involved the formation of collagen fibrils in vitro, the examination of the effect of proteoglycans on it and the direct determination of the interactions by a solid phase assay. The resistance of these interactions in the presence of various salts and buffers was also studied. In addition, the interactions were examined after specific enzymatic degradation of the interactions to reveal their regions responsible for the interactions.

They used for the study: type I collagen from bovine achille's tendon - type II collagen from bovine nasal cartilage and pig laryngeal cartilage. Aggrecan, biglycan and decorin have been used as Proteoglycan, isolated from pig laryngeal cartilage Squid proteoglycan termed D1D1A, D1D1B and D1D2.

As results, collagens type I and type II formed fibrils after incubation at 37 °C for about 12 h after which the absorbance of the solutions at 400 nm remained constant (Fig 1). The experiment was then repeated in the presence of the various proteoglycans studied: The technique of absorbance at 400 nm, with and without proteoglycan is acceptable because it is the same one used by *Miksfk and Deyl* pour comparer aortal and skin collagen pigmentation measured by absorbance at 350 nm (Miksfk and Deyl, 1991). One measures the appearance of

collagen; the more collagen is formed, the more absorbance is high. The results show as the author indicates that:

- Collagen type I fibrils formation was inhibited by decorin, D1D1A and D1D2 and
- Collagen type II was inhibited only by biglycan (Fig 1A, 1B). Thus, Biglycan interacts strongly with collagen type II and not with type I – Decorin, squid proteoglycan D1D1A and D1D2 interact with collagen type I, not with type II. This kind of investigation must be confirmed by several different methods as in the case of a protein assay. They therefore subjected their sample to the test of immunochemical quantification. The immobilised collagen was subjected to interaction with the various proteoglycans used in a wide range of concentrations and their amounts bound unto collagen were quantitated immunochemically. Here, it's the associative complex which is quantified: so absorbance depends to interaction, hence the change in the wavelength measurement at 492 nm (Fig 2) ; Wavelength is increased because the complex is larger. The results show as the author suggests, that:
 - Collagen type I was found to interact strongly with decorin, D1D1A and D1D2,
 - Very small extent with the squid proteoglycan population D1D1B, and
 - Collagen type II interacted only with biglycan (Fig 2).

The effect of various agents on the interactions of proteoglycans with collagen was then examined. This test is necessary since these agents describe the reality of the physiological medium. They therefore reproduced the initial test, varying the concentration of the agent. The results show that interactions were Lowered by the phosphate concentration, and also NaCl concentration (Fig 3).

The effect of proteoglycan core protein was then examined showing increasing of interactions with core protein than in simple proteoglycan in the case of Biglycan (Fig 4A). The author argues therefore that glycosaminoglycans don't contribute to the interaction because of the remarkable same dissociation constant with or without protein core (Fig 4B). In fact the K_d is strongly dependant to the interactive molecules presents in the middle. Thus if the K_d remains invariable, that means that GAG doesn't plays any role here. This is in agreement with the 2nd model of interaction proposed by the 3rd article (Fig 4C, 4D). On the other hand, none of the squid proteoglycan core interacts with collagen: in accordance with the hypothesis that the interactions are electrostatics and mediated by the GAG chain. This is in accordance with the 1st model proposed by the 3rd article (Fig 4A, 4B). All this justify that the interaction depends on the structure of the proteoglycan.

The source of collagen type, i.e. vertebrate or invertebrate, did not affect the interactions examined. Knowing that the main difference of these collagen molecules is the number of carbohydrate moieties, which is greater in the invertebrate collagen; this means that the carbohydrate part of the collagens is not involved in the interactions.

The author concludes by saying: « From the results of the present study it might be proposed that the various collagen molecules behaved differently in their interactions with the interstitial proteoglycans, most likely due to differences in the amino acid sequence ». It's a good assumption that the proteoglycan sugars tend to interact with amino acids of collagen.

This is the fundamental principle of microbial interactions: bacteria to infect cells send their peptide motifs sugars in the epithelium of the host that are specific, and it is from here that were born Glycodendrimers (Turnbull and Stoddart, 2002), (Shiao and Roy, 2012).

Mechanical interactions between collagen and proteoglycans stabilising soft tissue

Collagen and Elastin are thought to dominate the elasticity of the connective tissue including lung parenchyma. The glycosaminoglycans on the proteoglycans may also play a role because osmolarity of interstitial fluid can alter the repulsive forces on the negatively charged glycosaminoglycans, allowing them to collapse or inflate, which can affect the stretching and folding pattern of the fibers. Thus, in the 2nd paper, the author hypothesized that the elasticity of lung tissue arises primarily from the mechanical interaction between proteoglycans and fibers.

Authors want to demonstrate the important role of the proteoglycans is to stabilize the collagen-elastin network of connective tissues and contribute to lung elasticity and alveolar stability at low to medium lung volumes.

The author hypothesized that the electrostatically charged network of proteoglycans in which the collagen and elastin fibers are embedded resists the folding of the fibers and hence stabilizes the alveolar structure. When the concentration of positive ions is increased, the repulsive forces of the negative charges on the GAGs along the proteoglycans are “screened” and the proteoglycans collapse and decrease their resistance to deformation, which should result in a “softer” stress-strain curve. To test this hypothesis, they measured the stress-strain curves of normal lung tissue strips under conditions that increase or decrease the compressibility of the proteoglycan aggregates, i.e. hypotonic, normal, and hypertonic solutions.

The stress-strain curve depends on the salinity in each of the three solutions as Fig 1 shows, and according to statistics non-showed, sensitivity to osmolarity decreased after GAG digestion.

This paper looks like a review because most of the time, the author refers to someone else works, to argues comparing their own data mostly none shown. Thus based on the works performed by Al Jamal et al, using a variety of enzymes to prove that once a GAG is not linked to a protein core, a not charged like hyaluronic acid had minimal effects on mechanical properties. It is thus reasonable to conclude that it is the swelling of the charged GAGs on the proteoglycans that influences the macroscopic quasi-static mechanical properties of lung tissue strips. By increasing or decreasing the electrical repulsive forces in the GAGs, the proteoglycans become inflated or deflated, which shifts the stress-strain curve to the left or right, respectively (Fig 1).

The authors introduce parameter "r" measurement hypotonicity and repeat the previous experiment with several levels of concentration leading to the same result (Fig 3). They assumption behind their data none showed here is that osmolarity has a major effect on

the properties of the proteoglycans, but not on elastin or collagen. The effect of osmolarity on collagen is more complex because collagen has hydrophilic residues. The fixed-charge density of collagen is very little compared with proteoglycans, which in a dilute solution can occupy a domain as high as 1,000 times the volume of the chain in an unhydrated state. It seems likely that most of the change in stress that developed in the lung tissue because of a change in osmolarity was substantially influenced by the state of swelling of the proteoglycans. For the remainder, the authors use mathematical models that relate to the same idea throughout the article. This is the most boring of 3 items and looks almost to a journal.

Structural interactions elucidated by 3D Electron microscopy

The cornea, which contains proteoglycans with keratan sulphate or chondroitin / dermatan sulphate GAG chains, is an excellent model system in which to study collagen-proteoglycan structures and interactions. In this last paper, most explicit, most interesting of three papers, the authors performed a three-dimensional electron microscopic reconstruction of the cornea.

Based on data we will go through, authors propose that the characteristic fibril arrangement in cornea is controlled by the balance of a repulsive force arising from osmotic pressure and an attractive force due to the thermal motion of the proteoglycans.

They used conventional two-dimensional micrographs of bovine cornea show PGs as filaments of electron-dense material both in transverse and longitudinal sections (Fig 1). This is followed by real space 3D reconstructions of transverse and longitudinal views, as the 2D Images provide no 3D information about collagen-PG interactions (Fig 2).

As result, some PGs extend between, and are in contact with two or more collagen fibrils; other PGs are shorter and occupy the space between adjacent fibrils; Some PGs that form bridges between fibrils bridge adjacent fibrils only tangentially, so that a PG chain often extends between more than two collagen fibrils.

A previous work underlines the short size of an individual PG not long enough to connect more than 2 or 3 fibrils. A possible explanation is that GAG chains from separate PGs might join together to form the long staining complexes we see in 3D. Moreover, it cannot be excluded that other proteins or molecules present in the cornea may mediate some PG associations by means of hydrogen bonds between the GAG chains, hydrophobic interactions. The authors continue by emitting a package of assumptions as logical one to the other. On this, the 1st model proposed (Fig. 4A, 4B) shows the presence of electrostatic interactions mediated by non-covalent antiparallel interactions GAG; they can conceivably break and reform repeatedly, concept that would lead to the idea of a “fluid” cornea, in which the relative positions between collagen fibrils are not fixed, and neither are the interactions between the extrafibrillar PG molecules.

Another model has been proposed was proposed showing the attraction exerted by the proteoglycan fiber protein with its central, opposing the charge repulsion exerted positive environment between the fibrils. This model perfectly describes a scenario conducive to the osmotic pressure of the medium resulting in attractive and repulsive forces acting together.

Conclusion

This last article proposes visual solutions to all the observations made in the two preceding articles. In this sense, the two models proposed conclude our study well: The structure of the proteoglycan plays a major role in its interaction with collagen fibers; depending on the type, it binds to collagen either from its central protein or from its peripheral GAGs. As for collagen, only its amino acids seem to play a role in the interaction.

References Papers

1. Demitrios H. Vynios, Nicoletta Papageorgakopoulou, Helen Sazakli, Constantine P. Tsiganos. **The interactions of cartilage proteoglycans with collagens are determined by their structures.** (2001) *Biochimie* 83 899–906.
2. Francisco S. A. Cavalcante, Satoru Ito, Kelly Brewer, Hiroaki Sakai, Adriano M. Alencar, Murilo P. Almeida, José S. Andrade, Jr., Arnab Majumdar, Edward P. Ingenito. and Béla Suki. **Mechanical interactions between collagen and proteoglycans: implications for the stability of lung tissue.** (2005). *J Appl Physiol* 98:672-679.
3. Philip N. Lewis, Christian Pinali, Robert D. Young, Keith M. Meek, Andrew J. Quantock, and Carlo Knupp. **Structural Interactions between Collagen and Proteoglycans Are Elucidated by Three-Dimensional Electron Tomography of Bovine Cornea.** (2010) *Structure* 18, 239–245.

Supplements papers

Ivan Miksfc and Zdenek Deyl. **Change in the Amount of s-hexosyllysine, UV Absorbance, and Fluorescence of Collagen With Age in Different Animal Species.** *Journal of Gertmtologv: Biological sciences* (1991). Vol.46. No. 3. B1 11-116.

Tze Chieh Shiao and René Roy. **Glycodendrimers as functional antigens and antitumor vaccines.** *NewJ. Chem.*, (2012). **36**, 324–339.

W. Bruce Turnbull_, J. Fraser Stoddart. **Design and synthesis of glycodendrimers.** *Reviews in Molecular Biotechnology* (2002). 231_255