Basic Collagen Info

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Abstract

This document summarizes some basic information about collagen, the form it takes in the cornea, and ways that it can be manipulated in vitro, with an eye towards attempting to align collagen gels by spinning them.

1 Collagen in the cornea

Collagen is a class of predominantly long fibrillar proteins prevalent throughout the body, particularly in regions of high tensile stress. It is by far the most abundant protein in the cornea, making up 71% of its dry weight,[1] and is the principle protein in the 500 μm thick stroma, which is the largest part of the cornea.[2] The stroma is remarkable for its impressive toughness and tensile strength, both of which are made possible by collagen.

1.1 Layout of collagen

According to the lattice theory, corneal transparency occurs due to the regular spacing of parallel collagen fibrils, which are 20-35nm in diameter and about 30nm apart from each other in a hexagonal lattice.[3, 4] Inside the cornea are 200 to 250 lamellae of parallel collagen fibrils, with each lamella orthogonal to the next. Keratocytes reside principally between these lamellae, and maintain the collagen.

1.2 Types of collagen in cornea

Over 15 types of collagen have been found. [5] Different types appear in different layers of the cornea. The stroma contains primarily type I (60 weight%) collagen, but type V (10%) and type VI (25%) are also present, along with trace amounts of other types.[1] The exact ratios vary significantly between species and between different developmental stages.[4]

Type V collagen exists in the same fibrils as type I, and its incorporation has been shown to reduce fibril diameter in vitro.[1, 4] Type V and I collagens are *fibrillar* collagens, while type VI does not fall into a normal classification,[4] but is thought to hold together Type I and V fibrils to form larger fibers.[6]

2 Physical characteristics of collagen

In brief, collagens are molecules with regions of repeating Gly-X-Y triplets. Specifically, fibrillar collagens are composed of molecules with long left-handed α-helix regions terminated by short non-helical ends. Three of these molecules come together to form a *tropocollagen* characterized by right-handed a triple helix. These, in turn, self-assemble into small fibrils with 67nm periodic banding, which then self-assemble into larger fibers.

2.1 Primary, secondary, and tertiary structure

All collagens have long regions of largely repeating triplets in which Glycine makes up every third residue, and proline and glycine, which are often changed to hydroxyproline and hydroxylysine, make up the majority of the rest.[5] Segments of repeating Gly-X-Y triplets form α-chains, while other regions

form other structures.. [5] Types I and V collagen are primarily a long, uninterrupted *alpha* chain with short non-helical ends. Type VI consists of shorter helical regions interrupted by small globular sites, which give the impression of beads on a string.[6]

2.2 Quaternary structure

A typical Type I collagen triple-helix (tropocollagen) contains two α1(I) molecules and one α2(I) molecule.[5] These molecules are similar in shape and dimensions, but differ somewhat in their amino acid sequences. Following secretion from the cell, the *propeptide* ends are enzymatically removed from the ends of each procollagen, turning it into tropocollagen. Tropocollagen is 300nm long and about 1.5nm in diameter. [5] Each molecule has a molecular mass of approximately 300,000. (This is the sum of the three constituent molecules.)

The conversion from procollagen to tropocollagen reduces the molecules' solubility 1000-fold. This reduced solubility causes them to precipitate and self-assemble into fibrils, roughly 10-300nm in diameter. [5, 1] These fibrils are strengthened by cross-linking between lysine molecules as described in detail by Alberts.[5] Fibrils, possibly with the assistance of other types of collagen, then aggregate to form larger collagen fibers with diameters of up to several microns. Characteristic 67nm banding is evident on both fibrils and fibers.[5]

3 Solubilizing Collagen

3.1 Pepsin solubilization

Pepsin greatly increases the solubility of types I-III collagen.[7] Unfortunately, pepsin removes the non-helical ends of the vast majority of collagen molecules. The effect of this is three-fold: initiation of fibril formation does not require a temperature increase; fibril formation is slower; fibrils are smaller and shorter; and the characteristic 67nm banding is less evident.[8]

Vitrogen collagen is pepsin-treated type I, and thus is lacking the short non-helical ends. Its concentration is 3.0mg/ml.

3.2 Acid and Salt solubilization

Acid, often acetic or hydrochloric, greatly increases the solubility of collagen. Conversely, salt causes the collagen to precipitate. Unlike pepsin solubilization, this method does not significantly alter the collagen. Chandrakasan et. al. detail a method to acid-solubilize Type I collagen using acetic acid, and precipitate it with NaCl.[9]

Unfortunately, cross-linked collagen never dissolves completely, so large multi-molecule crosslinked collagen polymers will always be present unless removed.[9]

The collagen used thus far in our attempts at acid solubilization is Sigma's *Collagen from bovine Achilles tendon*, which is simply cleaned Achilles tendon with soluble protein extracted with Na_2HPO_4, and with mucopolysaccharides extracted using KCl, as described by Einbinder and Schubert[10]. Sigma has not characterized the collagen, beyond confirming that it is "suitable as a substrate for collagenase digestion assays."

Collagen in the Achilles tendon is especially highly cross-linked and durable.[5] Unfortunately, cross-linking makes collagen largely immune to solubilization, so that the Achilles tendon collagen *may* not dissociate well into individual tropocollagen molecules.

Many studies use rat tail tendon collagen for solubilization, presumably because of its lower levels of cross-linking. Furthermore, Chandrakasan et al. report that rat tail tendon collagen contains "Intermolecular aldimine crosslinks that are readily opened at acid pH," and discuss the use of a "lathyritic agent" in the rats to inhibit crosslinking.[9]

4 Synthetic collagen structures

Collagen, once dissolved, can be precipitated to form fibers. For instance, Williams et. al. worked with creating collagen fibrils in an aqueous solution using non-pepsin-treated collagen, and found that well-ordered fibrils similar to those seen in-vivo could be created. They found that the solution *should contain*

phosphate, and that temperatures should be from 20 to 30^{o}C [11]

4.1 Gels

Some of the first collagen gels were made by Elsdale and Bard, who used a .1 wt% Type I collagen solution. The solution is obtained by dissolving rat tail tendon in acetic acid. By quickly and simultaneously bringing the solution to neutral pH, and to a significantly higher ionic strength, the collagen precipitates into fibrils as opposed to useless aggregates. In their experiments, the collagen ultimately formed roughly 200nm wide fibrils with the characteristic 67nm banding. Very fine fibrils form quickly upon precipitation, and then slowly combine to form the larger 200nm fibrils. Furthermore, Elsdale and Bard note that by leaning a setting gel at an angle, the mixture will run and produce somewhat aligned fibrils. [12]

Other experimentalists have also used rat tail collagen.[13] However, many more recent experiments do use the pepsin-treated Vitrogen 100 collagen.[14] Presumably, such gels would exhibit less banding and smaller fibrils than those using non-pepsin-treated collagen. Also, unlike acid-solubilized collagen gels, they may begin to set immediately without need for initial incubation.

Interestingly, Guido and Tranquillo have successfully induced *some* fibril alignment in collagen by letting gels set in strong (2-20T) magnetic fields. They then used birefringence measurements to assess the degree of orientation.[15]

5 Possible experiments

As has been previously suggested, one possible experiment is spinning collagen gels. There are a number of variables that we could look at. Non-pepsin treated collagen seems to have greater potential to form long, well-ordered fibrils like those seen in vivo, but Vitrogen 100 is far easier to obtain and (for better or worse) seems to gel more slowly and without needing an incubation period.

Different spinning rates and times, different delays between solution preparation and spinning, as well as multiple applications of collagen, may affect the ultrastructure. Also, phosphate concentration, temperature, and dilution may affect how well fibrils form, and type V collagen concentration could affect fibril diameter, although it's not certain which of these relationships apply to pepsin-treated collagen, non-pepsin-treated, or both. Perhaps the best approach is to spin a few Vitrogen gels with widely varying speeds and dilution factors, in different stages of gelation, and examine them in SEM and AFM.

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