Structure of collagen proteins

Collagen forms the majority of the protein that is found in mammalian organisms and constitutes 30% of the total protein mass of a human. By being used as a gibbet, collagen is utilized by body cells towards the molding of their surroundings. This eventually creates an atmosphere favorable for normal cell function as well as the development of the tissues. Apart from providing mechanical support, collagen have several ligands which improve the performance of factor receptors and integrins that can control cellular procedures such as cell union, cell migration commonly known as chemotaxis, remodeling of tissues, as well as the healing of wounds. Collagen comprises of between 25 and 35 percent of the total protein tissue present in a mammal’s body. The hair, connective tissues as well major connective tissues are made up of collagen. Collagen is structured into fibrous strands, precise to their role (Lamberg 226).

Collagen is broken down into several sub-units known as tropocollagen. A Tropocollagen has a form of a triple helix attached to a hydrogen bond to form a polypeptide chain. A sample Collagen is predominantly made up of amino acids. It has high levels of proline and glycine alongside hydroxylysine and hydroxyproline. Vitamin C is essential during the building up process of collagens, although it is not a direct constituent of the amino acid chain.

Currently, there are 29 known collagen types of fiber. The most prevalent in the body are the type 1, 2, 3 and 4. The type one collagen is present in all soft tissues inclusive of the internal organs, the bones and tendons as well as the skin. On the other hand, Type 2 collagen is found in the cartilage of the body structures while type 3 is common in reticular membranes and tissues. Additionally, Type 4 collagen is only found in the membranes of the cell

basement. If the formation of collagen happens inside the cell, then the process is known as vivo formation. In this process, three peptide chains appear in the ribosomes all along the rough endoplasmic reticulum. On the contrary, if the collagen is formed outside the cell, then the method of formation is known as vitro formation. According to this process, Collagen is produced in a laboratory through manual procedures. One collagen is made up of a chain of five small tropocollagen molecules. The protein strand is made up of the amino acid base. The staggered arrangement of Tropocollagen molecules permits them to adhere to adjoining strands and thus providing the fibers with additional strength (Murrieta 16).

By designation, a collagen molecule is made up three Î± – chains also known as polypeptide chains and contains on the least, one domain having a repeating Gly-X-Y sequence in all of the essential chains (Myllyharju and Kivirikko 26). At present, all vertebrates are made up of at least 27 collagen type’s each having 42 distinct Î± chain. A number of collagens make up homotrimers having three Î± chains whereas others have two or even three distinct Î± chains. The X and Y locations can contain any amino acid apart from glycine. Characteristically, proline is only available in the X spot with 4-hydroxyproline in the Y position. Whereas 4-hydroxyprolines are necessary for the solidity of the triple helix, glycines are essential for filling the three chains into a coiled-coil configuration. This formation is exemplified as a left-handed helix that is wound about a regular axis to form a triple helix with a one-dimensional right-handed superhelical pitch, producing the ultimate arrangement of a rope-like rod.

Collagen Types

With the aim of avoiding confusion, collagens are given roman numerals in the order that they have discovered. Whilst referring to the composition of a collagen, each of the three Î± chains is initially quantified for chain number (1, 2, or 3) and thereafter the type of the collagen is identified. For instance, Î±2 (I) refers to the second Î± chain is type I whereas Î±1 (II) refers to the first Î± chain is type II collagen.

Collagen division into families is made essentially by the apparatus and organization of matrix gathering. The following are the nine collagen families along with their respective types. “ fibril-forming (I, II, III, V, XI, XXIV and XXVII), fibril-associated collagens with interrupted triple helices (FACITs) positioned on the exterior of fibrils (IX, XII, XIV, XVI, XIX, XX, XXI, XXII and XXVI), hexagonal form (VIII and X), basement membrane forming (IV), beaded filaments (VI), affixing fibrils for basement membranes (VII), transmembrane domains (XIII, XVII, XXIII and XXV), and the family of type XV and XVIII collagens” (Kivirriko 123).

Definite collagens are articulated in a tissue definite approach, as depicted in types II, IX and XI that are set up almost entirely in cartilage, although type XVII is just found in skin hemidesmosomes. In addition, some collagen forms are ordinary in the majority of extracellular matrices, as in the case of type I. Furthermore, collagen fibrils that frequently comprise more than one kind of collagen. Such a type I collagen may also possesses smaller amounts of types III, V and XII. Additional heterogeneity in the super family may be as a result of unusual splicing of the records of several genes as well as the use of option promoters in a number of genes. Through the huge figure of

structurally distinct members of the super family involves being caught up in numerous biological functions (Kadler 124).

Collagen assembly

Most of the collagens have a similar formulation procedure that’s characteristically linked with type I. By starting inside the cell, three peptide chains are produced in ribosomes all along the Rough Endoplasmic Reticulum (RER). The chains formed are then referred to as preprocollagens and each one of them possess registration peptides on the end as well as a signal peptide. Upon completion, these peptide chains are then sent into the lumen of the RER somewhere they are slashed into their procollagen shapes.

Whilst in the RER, the chains progress to undertake a chain of efficient changes. Initially, the lysine as well as proline amino acids are hydroxylated, a procedure that depends on ascorbic acid. Subsequently, precise hydroxylated amino acids are glycosylated, permitting the three chains to relate into a triple helical formation. Lastly, the procollagen is transported to the Golgi apparatus for packaging as well as secretion in a process known as exocytosis.

The moment the collagen is outside the cell, it is again reordered into a functional matrix. listing peptides are sliced via procollagen peptidase, to form tropocollagen, which can which has the potential to aggregate itself and form collagen fibers. In the case of non-fibular collagen, the N- and C-propeptides remain in the cell where they assist in directing super molecular assembly. Following the formation of fiber, inter-chain cross-linking of

collagen take place between lysine and hydroxylysine residues subsequent to deamination from lysyl oxidase (Kivirriko 123).

Molecular Structure

A collagen molecule also known as tropocollagen is a sub-unit of bigger collagen collection as in the case of fibrils. The molecule has a diameter of around 1. 5nm and is 300nm long. It is made up of three polypeptide strand, each having left-handed helix conformation. In addition, the three left-handed helices are twisted collectively to form a right handed super helix, a joint quaternary structure alleviated by several hydrogen bonds. The association of type I collagen with possible fibrillar collagens to form a branded triple helix is referred to as microfibril. Every microfibril is interdigitated with its adjoining microfibrils to an extent that may propose that they are independently unbalanced even though within collagen fibrils they are so well structured to be crystalline.

Since glycine is the least amino acid having no side chain, it has a unique responsibility in fibrous structural proteins. In the formation of collagen, Gly is essential at all third position since the assemblage of the triple helix holds this residue at the inner (axis) of the helix, wherever there is no gap for a larger side group than glycine’s sole hydrogen atom. For similar basis, the rings of the Pro and Hyp should point outward. The function of the two amino acids is to help stabilize the triple helix.

Fibrillar Structure

The tropocollagen subunits impulsively assemble itself with recurrently spread out ends, into even bigger arrays in the extracellular vacant places of

tissues. In the case of fibrillar collagens, the molecules are spread out from each other by 67nm. Each and every D-period has 4 and fraction molecules of collagen. This is due to the fact that if you divide 300 by 67 doesn’t yield a large integer. Therefore in each D-period duplicated of the microfibril, there exists a part having five molecules in a cross-section known as overlap. On the other hand, the part consisting of four molecules is known as the “ Gap”. The triple-helices may also be prearranged in a hexagonal or quasi-hexagonal arrangement in section, both the overlap region and the Gap. Equally the gap and overlap regions (Xie 549).

There exists a covalent is cross connections in the triple helices, as well as an amount of covalent cross connections involving tropocollagen helices outlining a well structured collection like fibrils. Bigger fibrillar bunches are produced with the support of numerous diverse categories of proteins as well as diverse collagen brands, proteoglycans and glycoprotein to shape the diverse kinds of fully-grown tissues from alternating blends of the similar major players. The insolubility of collagens has been a barrier to the research carried out on monomeric until it was discovered that tropocollagen from immature animals could be removed since by then, is it not yet completely cross connected. Nonetheless, progress in microscopy systems microscopy of electron (EM) and “ atomic force microscopy (AFM)” and the diffraction of X-ray have facilitated those doing research to acquire gradually more comprehensive figures of collagen configuration in situ.

This afterward progress is predominantly significant to improved perception of the system in which collagen configuration influences communication in both intracellular and cell-matrix stages, and how tissues are build in

development and restoration, and altered in growth and infection. For instance by means of AFM -based nanoindentation, researchers have been able to show that a particular fibril of collagen is a varied substance alongside its axial course with extensively diverse automatic functions in its fissure and partly covered areas, connecting with its diverse molecular associations in these two areas.

The fibrils of collagen are partially crystalline collections of molecules made of collagen. Collagen fibers (filaments) are bunches of fibrils. Fibrils/ collections of collagen are prearranged in diverse arrangements and attentiveness in a variety of tissues to offer unstable tissue elements. In fillets, complete triple helices of collagen are positioned in a parallel, reeled display. Forty nm spaces involving the endings of the tropocollagen subdivisions-roughly equivalent to the breach section- almost certainly act as nucleation position for the deposition of long, hard, fine crystals of the mineral component, which is (approximately) hydroxyapatite, Ca10(PO4)6(OH)2 with some phosphate. It is in this way that certain kinds of cartilage turn into bone. Type I collagen gives bone its tensile strength.

Prolyl 4-Hydroxylase (P4H)

As formerly stated, hydroxylation of the Y-position proline residues is a critical modification for generating stable triple helical collagen. This modification is carried out in the lumen of the RER by the enzyme prolyl 4-hydroxylase (Tandon 199). The vertebrate forms of these P4H’s are Î±2 Î²2 tetramers in which the Î² subunit is identical to the protein disulfide isomerase PDI (Myllyharju, 2003). Various isoforms of the catalytic a subunit

have been found in organisms of varying size and complexity; from humans to Drosophila.

Another family of P4H’s in the cytoplasm has been uncovered and has been linked to the regulation of the hypoxia-inducible transcription factor HIF. Cytoplasmic P4H’s have no PDI subunit, require different sequences flanking the prolines that are hydroxylated, and have markedly higher Km values (Kivirikko and Myllyharju 199). No overall amino acid sequence homology is detected between the collagen and the cytoplasmic HIF P4H’s, with the exception of critical catalytic residues. HIF is continuously synthesized and under normoxic conditions a critical proline residue in a -Leu-X-X-Leu-Ala-Prosequence is hydroxylated by the cytoplasmic P4H’s, not by collagen P4Hs. The resulting 4-hydroxyproline residue is essential for HIFÎ± binding to the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex for subsequent proteasomal degradation. However, under hypoxic conditions hydroxylation ceases, allowing HIFÎ± to escape degradation and instead forms a stable dimer with HIFÎ² (Jaakkola, 2001). Once formed, the dimer is translocated into the nucleus and becomes bound to the HIF-responsive elements in a number of hypoxia-inducible genes, such as those for erythropoietin, vascular endothelial growth factor, glycolytic enzymes and even for the Î±(I) subunit of human type I collagen (Takahashi 200).

Illinois Institute of Technology biologist Joseph Orgel used the high-energy X-rays produced by the APS to examine the structure of collagen, a protein that composes more than a quarter of all protein in the human body and forms the principal component of skin, teeth, ligaments, the heart, blood vessels, bones and cartilage. In these tissues, collagen molecules pack

themselves into overlapping bundles called fibrils. These fibrils, which each contain billions of atoms, entwine themselves into collagen fibres that are visible to the naked eye (Xuyang 2760).

Scientists have known the basic molecular structure of collagen since the 1950s, when several different international groups of scientists discovered that it had a triple-stranded helical structure. However, researches had never before had the ability to study the structure of an entire fibril in the same way that they could study an individual collagen molecule, according to Orgel.

Orgel and his team performed diffraction studies on intact collagen fibrils inside the tendons of rat tails in order to understand just how the protein functioned within unbroken tissue. “ We tried to draw a highly accurate map of the molecular structure of tissues,” Orgel said. “ By doing so, we hope to transform a very basic understanding that we have of the molecular structure of tissue into a much more tangible form.”

Since the scientists kept the tendon tissue intact, they could see how the collagen molecule binds to collagenases, a class of enzymes which when working properly help to regulate the normal growth and development of animals but when malfunctioning can lead to the metastasis of cancerous tumors or rheumatoid arthritis. The visualization of this interaction could help drug developers to create an inhibitor to prevent the pathological action of the enzyme, Orgel said.

Previous studies of the structure of collagen had looked only at crystals of small fragments of the protein, so scientists had little idea of how it looked

within intact tissue. “ It’s impossible to get the information that we did by removing tiny chunks of the tissue,” Orgel said. “ We couldn’t obtain this data by single-crystal crystallography. This research was made possible only because of the BioCAT beam line provided by the APS.

Applications

Collagen has been extensively used in beauty surgical procedures, hemostats, mechanism coatings, recovery fluids, formulation recipients, tablets, cartilage rebuilding, medicine release, in addition to skin substitutes for patients with burns. However, both medical and cosmetic use is declining because most commercially available collagens are derived from bovine or porcine tissues. Mainly enriched in type I collagen, these preparations also contain small amounts of type III as well as other collagens that are difficult and expensive to remove from the desired material. Moreover, there is a high rate of allergic reactions from animal-derived collagens, causing prolonged redness. Using collagen derived from cows also poses the risk of transmitting prion diseases such as bovine spongiform encephalopathy (BSE). The scientific community also uses collagen in its studying its role in tissue development and disease. Extracting sufficient quantities of nontraditional or less prominent collagens is a costly and difficult task (Kadler 196).

A processed form of collagen commonly used is gelatin. Derived from denatured collagen, gelatin is composed of a mixture of collagen chains of different length, structure, and composition. This distribution depends on what type(s) of collagens are extracted, the extraction method, as well as the pH and ionic strength of the solution used for processing. Because

gelatin is a heterogeneous composition, especially in size and isoelectric point, the resulting products will inevitably have variable gelling and physical properties. This variability presents a significant challenge for medical applications where stability, safety, and control are necessary (Crissman 192).

Cheaply produced recombinant collagens and gelatins have the potential to alleviate many of the issues associated with animal derived versions. Given the large number of aforementioned applications there is also a large market in this area. Scalable technology is needed to make microbial expression of recombinant collagens a viable alternative to tissue extraction. Using microbes to engineer collagen allows for greater control over collagen synthesis and organization, which in turn increases the quality, consistency, and safety of collagen production. It would also provide an easy platform for introducing altered primary sequences into recombinant collagens.

Such genetic control over collagen structure is crucial in studying the impact of specific mutations on collagen structural hierarchical assembly and associated functions and also would allow for the creation of designer collagen-mimetic materials. Recombinant expression would also allow for the extraction of sufficient quantities of native collagen forms that are present at low levels which are otherwise mainly characterized at cDNA and genomic levels. This would allow for structural and functional analysis of these rarer collagens (Baneyx 114).

Biomaterials applications for collagens in hemostats, as skin substitutes, in cartilage reconstruction, and for drug delivery can benefit from the improved

purity of cloned sources of collagen. Purity in this case would include both reducing other extracellular matrix components that may be carried through the purification process leading to potential inflammatory responses, or bioburdens with potential impact on human heath, particularly neurological disorders due to prion concerns. Recombinant human collagen seems to avoid immune reactions previously described and is therefore more biocompatible. Recombinantly derived collagen was shown to have superior mechanical strength and haemostatic activity compared to animal derived collagen when formed into a matrix. They can be altered to include bioactive peptide sequences as well as to be collagenase resistant.

Recombinant gelatins can be tailored to alter their gelling temperature by controlling their hydroxyproline content. Moreover, they have been shown to be less allergenic. As they are widely used in the food and drug industry, recombinantly derived gelatins can be made animal-free and thus open for consumption by vegetarians (Baez 252).