CHAPTER TWO

INTRODUCTION TO GELATIN
CHAPTER - II

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Gelatin is a biopolymer. Gelatins are a class of proteinaceous substance that have no existence in nature, but derived from a parent protein, called collagen, by one of the many ways involving the destruction of secondary structure of the collagen and, in most of the cases, some aspects of primary and tertiary structures. Mostly this is achieved by both chemical and thermal treatments. This is the reason why it is sometimes called as denatured or disorganized collagen.

Collagen is the main protein constituent of the white fibrous connective tissues. They serve as the chief tensile-bearing elements for all mammals and fishes. Collagen comprises of 30% of the total organic matter in mammals and 60% of the total protein contents. Much of the collagen is localized in major tissues like skin, bones, tendons but collagen fibers pervade almost every organ and tissue. Due to its wide spread distribution and function, fibers in various tissues are organized macroscopically in different ways, are produced by different types of cells and are intimately associated with varying types and amounts of other substance. There are many ways a collagen can be converted to gelatin. So it will not be completely surprising if one gets gelatin of different nature and character, when extracted from different collagens by different methods. In fact we do get gelatin of different nature and character.

Gelatin is the only degraded protein that have excited so much of scientific interest. Originally because of its use in food (like: salads, candies, bakery goods, etc.), pharmaceutical and cosmetics industries. While, this is still true, of late much attention has been focused on gelatin to understand the biologically significant parent protein collagen, from the knowledge of basic properties of gelatin. Gelatin is also somewhat unique among all the proteins due to the absence of appreciable internal order. So in aqueous solution at sufficiently high temperature the peptide chains take up random configuration. This is analogous to the behavior of synthetic linear chain high polymers. So this allow us to examine
the structure and behavior of gelatin from the point of view of the theories developed to treat such high polymer systems. Another interesting area of study which has received considerable attention in the recent past is the sol-gel transition in gelatin gel. From this one can derive information about explicit nature of phase transition occurring at gelation point, properties of physical gels, scaling relations, etc.

Since collagen is a protein and the derived element gelatin (denatured collagen) is also a protein it is worth mentioning a few words here about the protein.

II.A WHAT IS A PROTEIN?

The name protein is derived from a Greek word proteios meaning of prime importance. Most of the solid matter from which living organism are made has a remarkably uniform composition. Only a few elements are present and they occur in proportion 54% of carbon, 7% of hydrogen, 16% of nitrogen, 22% of oxygen and 1% of sulphur. Clearly they are of great importance in the construction and chemical activity of living organism and are called as proteins. A protein is a linear chain whose back bone is made up of succession of amino-acid monomers, whose basic composition is,

\[ R - C - COOH \]

where COOH is the carboxyl group, \( NH_2 \) is the amine group and \( 'R' \) is the side group (radical) of the amino-acid. \( 'R' \) can contain, apart from \( 'C' \) and \( 'H' \) (Hydro-Carbon), other atoms like \( 'O' \), \( 'S' \), \( 'N' \), etc. There are twenty types of amino-acids which differ in their side groups. The smallest and simplest one being glycine, when \( R=H \). Under the normal physiological conditions the amino-acids exist in doubly ionized form. In this case the acidic carboxyl group can lose a proton and the basic amino group gain a proton to form a dipolar ion or zwitterion.
Having atomic groupings of both kinds, they exhibit properties of both carboxylic acids and amines.

II.B WHAT ARE PEPTIDES?

As said above, proteins are made up of many amino-acids. All these amino-acids are bound together by a peptide linkage formed between the amino group of one amino-acid and the carboxyl group of another. Proteins are formed by polycondensation of amino-acids. In each steps of condensation a peptide bond is formed by the loss of one water molecule. When two amino-acids combine this way, they form a single peptide bond and the resulting product is a di-peptide.

\[ \begin{align*} 
\text{R}_1 & \quad \text{R}_2 \\
\text{NH}_3^+ & \quad \text{C} \quad \text{COO}^- \quad \text{+} \quad \text{NH}_3^+ & \quad \text{C} \quad \text{COO}^- \\
\text{H} & \quad \text{H} \\
\downarrow & \quad - \quad \text{H}_2\text{O} \\
\text{NH}_3^+ & \quad \text{C} \quad \text{C} \quad \text{N} \quad \text{C} \quad \text{COO}^- \\
\text{H} & \quad \text{H} \quad \text{H} \\
\end{align*} \]

(a di-peptide-bond)

In this way, when 3 amino-acids combine together, they form two peptide bond and the resulting product is a tri-peptide. A tetra-peptide, a penta-peptide can be formed out of 4 amino-acids, 5 amino-acids respectively. When the number of peptide bond is less than 100 it is generally called as a poly-peptide and when it is more than 100, it is called as a protein, although the demarcation is not very sharp.

The characteristic molecular masses of individual poly-peptide chains in proteins are of the order of 20,000, which corresponds to 150-180 amino acid residues (the average molecular weight of an amino acid residue is 120).
The peptide bond - (CO - NH) -, which joins together amino-acids in protein, has a specific planar structure as has been found by x-ray structure analysis. All the four atoms in the peptide bond lie in one plane. The (N-C) bond is shorter than the same bond in di-phatic amines (R-NH₂), where its length is equal to 0.147 nm. The shortened length of (N-C) bond, as well as the planar arrangement of the bonds, is evidence of the conjugation of (N-C) and (C=O) bonds and hence, of the overlap of their electron shells, this being accompanied by shift of electron density from N to C. This is the reason why (N-C) bond is partially double and (C=O) bond is partially single, i.e.,

\[ \text{Fig. II.1 Figure of a peptide bond.} \]

**II.C CHEMICAL COMPOSITION OF A COLLAGEN**

Collagen is unique among the proteins because of its amino-acid composition. It is the only mammalian protein containing large amount of *hydroxyproline* and it is extraordinarily rich in *glycine* and *proline*. Hydroxyproline is almost unique characteristics of collagen. The sulphur contain of collagen is very low.

Every third element of collagen is a glycine residue (33%). And an important amount of amino-acid proline and hydroxy proline residues (25% of the total) is present in collagen protein. As discussed above, although the chemical composition of collagen protein is not unique, a typical sequence of collagen protein is given by [Perzon, I. and Djabourova, M., 1990],

\[ - (\text{Gly} - \text{Pro} - \text{X}) - \quad \text{OR} \quad - (\text{Gly} - \text{X} - \text{Hypro}) - \]

where 'X' being different amino acids.
The presence of these rings in proline and hydroxy-proline give an enhanced localized rigidity to the chain. The side groups of the amino-acids also play an important role in the stability of proteins. Some of them have polar groups (like: OH, CO, NH$_2$, etc.) which are likely to interact with water molecules and establish hydrogen bonds. There are also polar charged groups (like: NH$_3^+$, COO$^-$, etc.) in variable amounts. The portion of the charged and uncharged groups varies with pH; on NH group for example, may be either NH$_3^+$ or NH$_2$. Charged groups are also hydrophilic. Hydrophobic groups (such as those of proline) are also encountered. Thus the conformation that a protein adopts when it is dissolved in solution (generally aqueous solution) is a direct consequence of the balance between hydrophilic and hydrophobic interaction, which in turn results from molecular composition [Djabourov, M., 1988].

The total length of a collagen strand is 1000 residues (primary structure). The individual chains are twisted in left handed helix which have 10 residues per 3 turns. The pitch of the helix is approximately 0.9 nm. The torsion of the chain is such that (C=O) and (N-H) groups attached on the main chain are oriented
perpendicular to its axis and are not in position to establish intra-chain hydrogen bond to stabilize the helix. So the question arises, how else could the helix be stabilized? The answer for collagen is the triple-helix. The three strands are wrapped into a super right handed helix, with a pitch roughly 10 times longer (~ 8.6 nm). The presence of 'Gly' is required to allow the three chains came close to each other, while 'Pro' and 'Hypro' residues enhance the rigidity. The gradual gentle right handed twist of the individual strands allow the side groups of various sizes to come into the structures.

The collagen triple-helix is stabilized by inter-chain hydrogen bonds which are perpendicular to the chain axis. The hydrogen bonds can be of several types, either directly between (C=O) and (N-H) between two adjacent backbone or via water molecules situated in interstitial positions inside the triple-helix. The overall length of the rod of triple-helix is around 300 nm. The rods are arranged in parallel rows, to build the fibers which are attached by additional covalent bonds located at both ends of the rods. These bonds makes the collagen fibers insoluble. The structure of collagen molecules at different levels are shown in Fig.(II.2)

Fig.II.2 Levels of organisation of a collagen : (a) the collagen fibril showing the staggered arrangement of collagen molecules; (b) the collagen rod; (c) enlarged details of the triple-helix; (d) the single chain; (e) the chain composition.

II.D COLLAGEN TO GELATIN TRANSITION

Collagen is extracted from tissue by chemical (acidic or alkaline) and thermal treatments. The main aim is to break the covalent bonds between the rods and to
separate the individual chains. This treatment also denatures the triple-helix by extensively breaking the hydrogen bonds which maintain it. Undesired mineral or organic components are also eliminated in this process. From all these processes the resulting material is gelatin. The transition from collagen to gelatin is sharp and complete within few minutes over a small temperature interval. The activation energy for denaturation is \( \sim 81 \text{ Kcal} \) and entropy of activation is \( +230 \text{ e.u.} \). The disordered molecule can fall apart in one of three ways as shown in Fig.(II.3). If

![Fig.II.3 A schematic diagram of the modes of conversion of monomeric collagen to various types of gelatin, assuming no rupture of the peptide bonds.](image)

there are no additional restraining bonds between chains (path-1), three randomly coiled single strand peptide chains result. The three chains may not have identical composition and probably not of equal molecular weight. These chains are called \( \alpha \)-chains. In the second case (path-2) two chains are joined together by one or more covalent cross-linkages. In this case the denaturation occurs in such a way, that leads to appearance of two chains; one \( \alpha \)-chain, the other a two stranded molecule having molecular weight approximately twice, that of the \( \alpha \)-chains; called as \( \beta \)-chain. \( \beta' \) component may be composed of two similar or unlike \( \alpha \)-chains. The weight distribution will be 67% of \( \beta' \) and 33% of \( \alpha' \). In the final case (path-3), atleast two covalent cross-linkages hold the three chains together. The disordering process melts out all the traces of secondary structure, but the three chains remain as a unit in solution. This three chain structure is called the \( \gamma \)-component.
The ideal conversion of the collagen monomer to gelatin should proceed along path one. So the number average molecular weight of the gelatin system should be one third the molecular weight of the collagen monomer and the weight average molecular weight should be little bit higher due to nonidentity of the chains. The best value of collagen monomer molecular weight is substantially higher than 300,000 Daltons. So the minimum molecular weight of the parent gelatin must be greater than 100,000 Daltons. In any real case, where intra-molecular polymerization is a factor, both minimum and maximum should be substantially greater than 100,000 Daltons [Veis, A., 1964].