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Theoretical study of temperature induced transition and hyper stability of collagen mimics

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Abstract

Collagen a polymer, is the nature's most abundant protein. It has an immense tensile strength and is the main constituent of ligaments, tendons, etc. The present communication interprets the experimental data of thermally induced transition in collagen mimics as reported by Steven K. Holmgren and co-workers. The theoretical transition curves as obtained by the modified Zimm and Bragg model are found to be in good agreement with the experimental data. The order of the values of nucleation parameter and enthalpy changes obtained theoretically, attributes the increase in the degree of the stability of collagen mimics [(Pro–Pro–Gly)$_{10}$⟨(Pro–Hyp–Gly)$_{10}$⟨(Pro–Flp–Gly)$_{10}$].

Keywords: Collagen; Nucleation parameter; Stability

1. Introduction

Collagens constitute a super-family of structural proteins of the extra-cellular matrix. There are hardly few other proteins in the nature with such regularity. The sequence in each chain of the collagen is predominantly repeats of (X–Y–Gly), in a left handed helical chain and these polypeptide chains in turn form a right handed triple helix. The first and the second positions of the repeat are more oftenly occupied by L-proline [Pro] and 4(*R*)-hydroxy-L-proline [Hyp], respectively. The expression of collagens in all tissues of the living organism has made the collagen a distinct/novel model of great interest from the viewpoint of molecular dynamics of polypeptides and proteins [1–16]. Steven K. Holmgren and co-workers experimentally studied the temperature induced transition in collagen mimics and they found that the stability of (Pro–Flp–Gly)$_{10}$ far exceeds the stability of any other known collagen of similar size [17].

The present communication explains the phenomenon of temperature induced transition in the various collagen mimics and their relative thermal stabilities, using the modified Zimm and Bragg model of helix ↔ coil (order ↔ disorder) transition, as reported in our recent publications in this journal [18,19], where the Zimm and Bragg model [20] was modified to explain the temperature induced transition in poly (β-benzyl-L-aspartate) [PBLAsp] [18] and in it's copolymer as well as pressure induced transition in polystyrene–polybutadiene [PS–PB] [19], a synthetic diblock copolymer.

2. Theory

The collagen has been treated here as two phase system. The Zimm and Bragg model for helix ↔ coil (order ↔ disorder) transition [20] has been modified to explain the thermal hyper-stability of the collagen mimics. An expression for degree of order Q is obtained from the grand partition function for entire chain in terms of nucleation parameter. Taking into account the nearest-neighbour interactions, the basic transition matrix M is given below.

$$M = \begin{array}{c} \\ h \\ r \end{array} \begin{array}{ccc} h & r \\ 1 & \sigma s \\ 1 & s \end{array}$$

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where σ and s are the nucleation and growth parameters and h and r are, respectively, the segments in ordered and disordered regions of the macromolecular triple helical chain.

The eigenroots of M, determined by the secular equation

$$|M-\lambda I| = 0 \tag{1}$$

are as follows:

$$\lambda_1 = (1/2)[(1+s) + \sqrt{\{(1-s)^2 + 4\sigma s\}}] \tag{2}$$

$$\lambda_2 = (1/2)[(1+s) - \sqrt{\{(1-s)^2 + 4\sigma s\}}] \tag{3}$$

The matrix formalism leads to the following, diagonalizing T and T^{-1} matrices

$$T = \begin{matrix} 1 & 1 \\ 1/[\lambda_1 - s] & 1/[\lambda_2 - s] \end{matrix}$$

and

$$T^{-1} = \begin{matrix} A_1 & A_1[\lambda_1 - 1] \\ A_2 & A_2[\lambda_2 - 1] \end{matrix}$$

Where the values of A_1 and A_2 are given by

$$A_1 = [\lambda_1 - s]/[\lambda_1 - \lambda_2], \qquad A_2 = [\lambda_2 - s]/[\lambda_2 - \lambda_1]$$

The growth parameter s is given by the following expression

$$s = \exp[(\Delta H/R)(1/T - 1/T_f)] \tag{4}$$

where T_f is the transition temperature.

The partition function Z for a chain of N segments is

$$Z = C_1\lambda_1^N + C_2\lambda_2^N = Z_1 + Z_2 \tag{5}$$

The value of coefficients C_1 and C_2 are given by

$$C_1 = \sigma s/[\lambda_1 - \lambda_2], \quad C_2 = \sigma s/[\lambda_2 - \lambda_1]$$

Hence the fraction of segment in ordered state

$$Q = [1/N][\partial \ln Z/\partial \ln s]$$

$$Q = [\{1 - A_1(1+B)\}/(1-B)] + [\{(1 - s + 2\sigma s)\}/N(\lambda_1 - \lambda_2)^2] \tag{6}$$

where $B = [\lambda_2/\lambda_1]^N$.

3. Results and discussion

The most defining feature of the melting/denaturation of collagens is that, on increasing the temperature above a certain critical value, its regular structure breaks down with the separation of all the three constituent chains, and adopts the random coil state as represented by the following step:

$[\alpha]_3$ (helical state) $\leftrightarrow 3[\alpha]$ (random coiled state)

We report here an extension of the Zimm and Bragg model to explain the temperature induced helix $\leftrightarrow$ coil transition in collagen mimics. Since the collagen mimics (model polypeptide) under consideration are of very small length [$N = 10$], the end effects and the inter-stranded interactions have been taken into account simply by considering all the three chains, each of N segments, arranged simultaneously in a sequence.

In the transition region the curves are found to be linear. The melting temperature depends on the sharpness of the transition curve. The value of enthalpy changes, and the fluctuations around the transition point control the sharpness of the transition. The holistic effect of these is reflected in the magnitude of σ and the half width of transition profile. The smaller the value of σ, sharper is the transition.

For all the theoretical purposes the value of nucleation parameter for a given collagen mimic is assumed to be constant for the entire range of temperature as well as independent of other surrounding interactions, whereas the growth parameter s has a very strong dependence on the temperature.

The value of σ in case of $(Pro–Hyp–Gly)_{10}$ is theoretically found to be smaller than the $(Pro–Pro–Gly)_{10}$ case, indicating the fact that the nucleation of latter is easier. The growth of the $(Pro–Hyp–Gly)_{10}$ is relatively difficult as compared to the $(Pro–Pro–Gly)_{10}$, which is because of the fact that the change in enthalpy as obtained theoretically is larger in former than the latter (refer to Table 1). This results in a higher thermal stability of collagen mimic $(Pro–Hyp–Gly)_{10}$ and is manifested by the higher transition temperature of $(Pro–Hyp–Gly)_{10}$ as compared to $(Pro–Pro–Gly)_{10}$ case (refer to the Table 1). This fact is well reflected in slope of transition curve (refer to Figs. 1 and 2).

The nucleation parameter σ, which provides the best fit between the theoretical and experimental data is found to be the lowest for the case in which the hydroxyl groups are substituted by fluorine atoms in 4*R*-Hyp residues. The order of the values of nucleation parameter σ obtained theoretically i.e. [$\sigma(Pro–Pro–Gly)_{10} \rangle \sigma(Pro–Hyp–Gly)_{10} \rangle \sigma(Pro–Flp–Gly)_{10}$], is in line with the commensurate increase in the degree of the stability of corresponding collagen mimics [$(Pro–Pro–Gly)_{10} \langle (Pro–Hyp–Gly)_{10} \langle (Pro–Flp–Gly)_{10}$], as reported by Holmgren et al. These theoretical observations as reflected in the transition

Table 1
Transition parameters for the temperature induced transition in collagen mimics

S. no.	Type of collagen mimic	Electro-negativity of substituted element at position Y in repeat unit of collagen mimics [21]	Transition temperatures (K)	Transition enthalpy ΔH (kcal/mol)	Nucleation parameters
1	$(Pro–Pro–Gly)_7$	–	279	03.5	9.0×10^{-4}
2	$(Pro–Pro–Gly)_{10}$	–	314	12.0	1.5×10^{-4}
3	$(Pro–Hyp–Gly)_{10}$	3.5 for oxygen atom in hydroxyl group in [Hyp] residue	342	15.0	5.1×10^{-5}
4	$(Pro–Flp–Gly)_{10}$	4.0 for fluorine atom substituting the hydroxyl group in Hyp residue resulting in [Flp] residue	364	20.0	6.4×10^{-6}

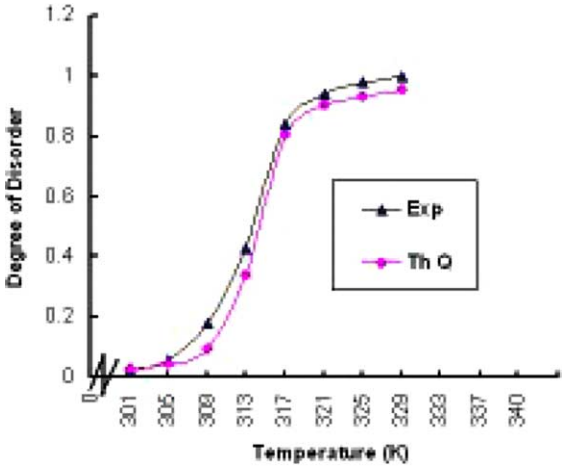

Fig. 1. Schematic representation of degree of disorder as a function of temperature in $[Pro–Pro–Gly]_{10}$.

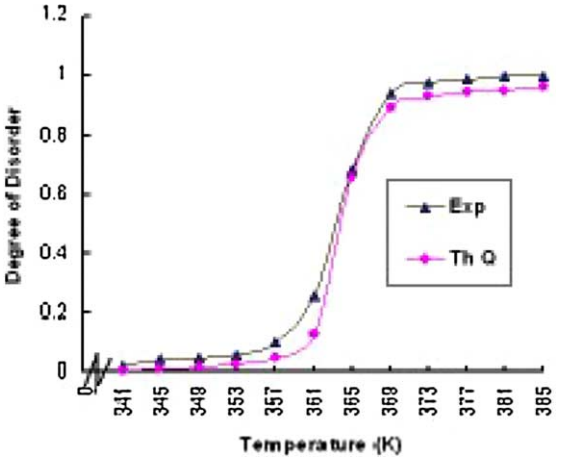

Fig. 3. Schematic representation of degree of disorder as a function of temperature in $[Pro–Flp–Gly]_{10}$.

region (Figs. 1–3), also correlate distinctly with the thermal-stabilities of the collagen mimics. The comparative degree of stability is further manifested by the shift in transition temperature T_f, i.e. $T_f(Pro–Flp–Gly)_{10} \rangle T_f(Pro–Hyp–Gly)_{10} \rangle T_f$ $(Pro–Pro–Gly)_{10}$. Due to the fact, that smaller the value of σ, the larger will be the free energy penalty in creating the transition/boundary interface [22], the higher values of change in enthalpy ΔH arrived at theoretically, may well be attributed to the increasing degree of stability of collagens. The introduction/substitution of the different atoms/groups at the second position of the repeat in the collagen mimic results in the increased thermal-stability in the order of their corresponding electro-negativities of the atoms/group concerned. A very sharp decrease in the values of the nucleation parameter with the simultaneous increase in enthalpy changes has been observed theoretically (refer to Table 1), corresponding to the increase in the value of electro-negativity of the atoms/groups [21] at the second position in collagen mimics (hence the increase in transition temperature as reported by Holmgren). All these facts indicate the increase in stability of the collagen mimics (refer to Table 1).

The same model has been employed successfully to explain the temperature induced transition in $(Pro–Pro–Gly)_7$ [23], another collagen mimic of shorter chain length. The value of σ in case of $(Pro–Pro–Gly)_7$ is theoretically found to be higher than the $(Pro–Pro–Gly)_{10}$, whereas the change in enthalpy in $(Pro–Pro–Gly)_7$ is found to be much smaller than $(Pro–Pro–Gly)_{10}$. These theoretical observations are consistent with the transition temperatures $T_f(Pro–Pro–Gly)_{10} \rangle T_f(Pro–Pro–Gly)_7$ (refer to the Fig. 4 and Table 1).

Although a considerable amount of effort has been employed in case of the collagens, towards the thermodynamical characterization of helix ↔ coil (order ↔ disorder) transition. But the actual mechanism that renders the collagens, the hyper-stability, is not yet completely understood. Ramchandran et al. and few others like Bella et al. [6–16] have tried to explain higher stability and the larger value of enthalpy in collagens as compared to other proteins, through

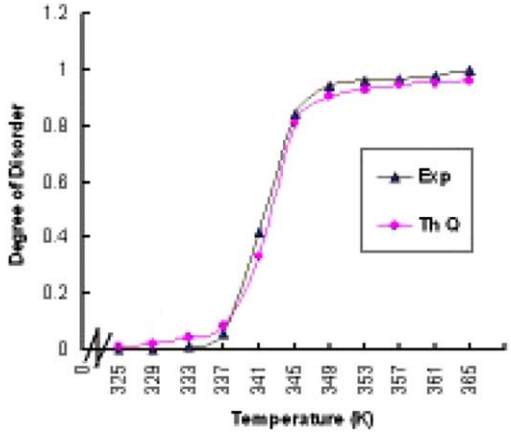

Fig. 2. Schematic representation of degree of disorder as a function of temperature in $[Pro–Hyp–Gly]_{10}$.

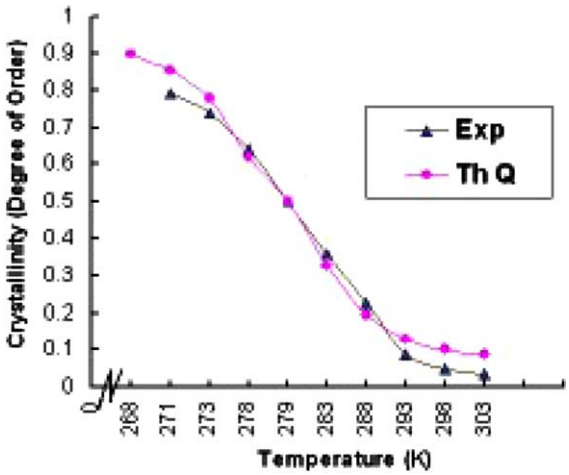

Fig. 4. Schematic representation of crystallinity as a function of temperature in $[Pro–Pro–Gly]_7$

the water bridges hypothesis. The recent work of Holmgren and co-workers suggests that the stability in collagen is not due to the inter-stand hydrogen bonds mediated through water bridges (as this mechanism entails collagens to pay a high entropic cost for immobilizing the water molecules). According to them the conformational stability in collagen arises on account of previously unappreciated inductive effects in [Hyp] as well as [Flp] residues.

The network of water bridges/entropic effect plays a major role in stabilization of the long chain natural collagens as observed by Ramchandran and others [6–16]. Whereas in the present communication, the Zimm and Bragg model for helix ↔ coil transition has been suitably amended to explain all aspects of the temperature induced transition including the hyper-stability, in the short synthetic collagen mimics, which derive their stability from the inductive effect [24–27]. Therefore, this theoretical work lends support to the conclusion that in case of the short chain synthetic collagens, the hitherto dominant entropic force loses it's significance and gives way to the more subtle inductive effect which is generated through the electro-negative atoms/groups, in their constituent residues ([Hyp] and [Flp]) of the collagen mimics, respectively.

4. Conclusions

As a further extension to the present day understanding of the stability/instability of collagens, one needs to synthesize and thermodynamically characterize the collagens of various types and varying sizes, in order to gain unique insight into the forces, responsible for hyper-stability of the collagens, arising due to the inductive and/or entropic effects. These effects are manifested during the helix–coil transition of the collagens and provide information about the stability and temperature dependent melting behaviour of the collagens. This kind of study in future, will not only resolve the paradox of mechanism which renders the hyper-stability to the collagen of any type and size, but will also usher the development of a completely new range of stable collagen based synthetic materials, specially the bio and bio-medical.

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