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Stereo-stability and temperature induced transitions of collagen mimics

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Abstract

Collagenous matrix proteins present in almost every part of the vertebrate organism and in many non-vertebrates are expressed in a rich structural variety. The present communication attributes the theoretical interpretation of temperature induced transitions in collagen mimics in terms of stereochemistry, as explained by the modified Zimm and Bragg model. The results are found to be in good agreement with the experimental data as reported independently by Steven K. Holmgren, Jonthan A. Hodges and Cara L. Jenkins. The order of the values of nucleation parameter and enthalpy changes obtained theoretically, is attributed not only to the stereo-chemistry but also to the relative increase in the degree of stability of collagen mimics.

Keywords: Collagen; Nucleation parameter; Stability

1. Introduction

In our previous publication [1] on temperature induced transitions in collagen mimics, the ability of our model to explain the failure of $[\text{Pro-hyp-Gly}]_N$ and $[\text{Pro-flp-Gly}]_N$, where hyp is 4*S* hydroxyproline and flp is 4*S* fluoroproline to form a stable triple helix was queried and was addressed to satisfactorily in short by the authors. The present communication explains in detail, the phenomenon of temperature induced transition in the collagen mimics and their relative thermal hyper-stabilities in terms of their ability/failure to form a stable triple helix, on account of the stereochemistry at C^{γ} position of proline, as manifested by the ability of $[\text{Pro-Hyp-Gly}]_7$, $[\text{Pro-Flp-Gly}]_7$, $[\text{Pro-Hyp-Gly}]_{10}$, $[\text{Pro-Flp-Gly}]_{10}$ and $[\text{flp-Pro-Gly}]_7$ to form a stable triple helix, whereas the failure in case of $[\text{Pro-hyp-Gly}]_{10}$, $[\text{Pro-flp-Gly}]_{10}$, and $[\text{Flp-Pro-Gly}]_7$ to form a stable triple helix using the same modified Zimm and Bragg model of helix ↔ coil (order ↔ disorder) transition [2]. Earlier, the model has been applied successfully to explain the temperature induced transition in poly(β-benzyl-L-aspartate) [PBLAsp] and in its copolymer and the pressure induced transition in polystyrene–polybutadiene, a di-block copolymer [3,4] and in few other important polymeric systems by the group [5–7].

Collagenous matrix proteins present in almost every part of the vertebrate organism and the marked diversity of its structure and tissue specific-functions has made the collagen mimics a distinct/novel model of great interest from the viewpoint of molecular dynamics of peptides/polypeptides and proteins [8–23]. It has the repetitive sequence X-Y-Gly, where X and Y are frequently proline and hydroxyproline. It forms a three-stranded triple helix with each helix (Fig. 1(a)) in a conformation similar to left-handed poly-proline II. The prolines at the X position provide elasticity and strength, due to the limited conformations it can assume and the glycines are too close to the other strands of the helix to allow room for a side chain. Many of the prolines at Y position are hydroxylated at the 3 and 4 positions of the proline ring that requires vitamin C. This modification occurs before the collagen is folded into a three-stranded helix (Fig. 1(b)) and thus stabilizes the triple helix by inter-chain hydrogen bonds.

In this communication, the experimental data reported independently by Holmgren et al. [24], Hodges et al. [25] and Jenkins et al. [26] have been used to explain the formation/failure of different stereo-isomeric forms at C^{γ} position of proline in collagen mimics.

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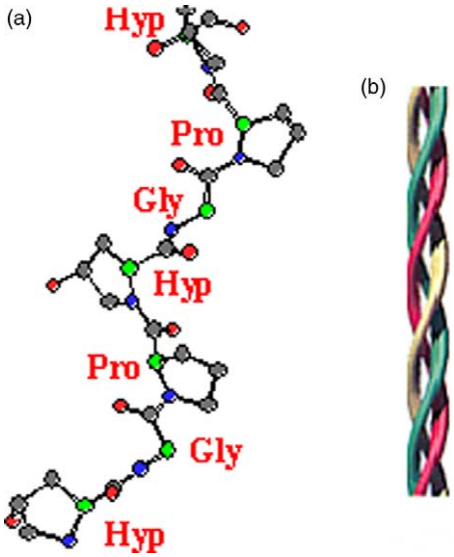

Fig. 1. (a) Left-handed α helical single strand of (Pro-Hyp-Gly)$_N$. (b) Right-handed collagen triple helix.

1.1. Theory

The collagen has been treated here as two phase system. The detailed theory of Zimm and Bragg model for helix↔coil (order↔disorder) transition modified to explain the relative stability of the collagen mimics has already been published [1]. 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.

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

The eigenroots of M, determined by the secular equation

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

are as follows

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

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

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 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

$$Z = C_1\lambda_1^N + C_2\lambda_2^N$$

Hence, the fraction of segment in ordered state

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

$$Q = [\{1 - A_1(1+B)\}/(1-B)] + [\{(1 - s + 2\,\sigma s)\}/N(\lambda_1 - \lambda_2)^2] \tag{5}$$

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

2. Results and discussion

We report here an extension of the Zimm and Bragg model to explain the temperature induced helix↔coil transition in collagen mimics having different lengths and their relative stability on the basis of the stereo-electronic effect/stereochemistry. Since, the collagen mimics (model polypeptide) under consideration are of very small length [N=7 and 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.

The thermal stability of the collagen triple helix is governed both by the position of the residue and the stereo-chemistry of the residue. The stereo-chemical as well as the positional effects of γ-substitution of proline are illustrated by the stabilities of [4(S)Flp-Pro-Gly]$_7$, [Pro-4(R)Hyp-Gly]$_7$, [Pro-4(R)Flp-Gly]$_7$, [Pro-4(R)Hyp-Gly]$_{10}$, and [Pro-4(R)Flp-Gly]$_{10}$, (increased triple helix stability). The positional effects become even more dominant with the increasing electro-negativity of the substituent atom/group in the concerned residue (refer to Table 1).

According to theoretical calculations based on the present modified Zimm–Bragg model, if the order of values of enthalpy change(s) ΔH in case of 4S hydroxyproline (hyp), 4R fluoroproline (Flp) and 4S fluoroproline (flp) are

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 [28]	Transition temperature in Kelvin	Transition enthalpy ΔH in K Cal/mol	Nucleation parameter
01	[4(S)Flp-Pro-Gly]$_7$	4.0 for fluorine atom in [flp] residue	306	09.0	6.0×10^{-4}
02	[Pro-4(R)Hyp-Gly]$_7$	3.5 for oxygen atom in [Hyp] residue	309	09.1	2.0×10^{-4}
03	[Pro-4(R)Flp-Gly]$_7$	4.0 for fluorine atom in [Flp]	318	12.6	8.2×10^{-5}
04	[Pro-4(R)Hyp-Gly]$_{10}$	3.5 for oxygen atom in [Hyp]	342	15.0	5.1×10^{-5}
05	[Pro-4(R)Flp-Gly]$_{10}$	4.0 for fluorine atom in [Flp]	364	20.0	6.4×10^{-6}

Table 2
Transition parameters corresponding to the failure of temperature induced transition in collagen mimics

S. no.	Type of collagen mimic	Transition enthalpy ΔH in K Cal/mol	Nucleation parameter 'σ'
01	[4(*R*)Flp-Pro-Gly]$_7$	09	>1
02	[Pro-4(*S*)Hyp-Gly]$_{10}$	15	>1
03	[Pro-4(*S*)Flp-Gly]$_{10}$	20	>1

assumed to be the same as in the cases of 4*R* hydroxyproline (Hyp), 4*S* fluoroproline (Flp) and 4*R* fluoroproline (flp), respectively (refer to Tables 1 and 2), then the substitution of the Flp at the first position and hyp and flp at the second position in [Pro-Pro-Gly]$_7$ and [Pro-Pro-Gly]$_{10}$ collagen mimics, theoretically lead to the arrival of highly unrealistic values of nucleation parameter σ, i.e. ($\sigma > 1$). The σ values thus obtained are several orders of magnitude higher than the σ values of the corresponding stable collagen mimics and for all the other known natural as well as synthetic polymers. The situation in which the value of σ is greater than one, is highly characterized by the complete lack of cooperativity [02], and reflects the failure to form a stable helical structure according to the Zimm–Bragg model (refer to Table 2). Hence in such types of situations, the inability to form a stable triple helix, in cases of [Pro-hyp-Gly]$_{10}$ and [Pro-flp-Gly]$_{10}$ and [Flp-Pro-Gly]$_7$ has been observed and is true in general for [Pro-hyp-Gly]$_N$ and [Pro-flp-Gly]$_N$ and [Flp-Pro-Gly]$_N$.

For all the theoretical purposes the value of nucleation parameter σ 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 nucleation parameter σ (the smaller the value of σ, higher is the cooperativity), which provides the best fit between the theoretical and experimental data are listed in Table 1. The order of the values of nucleation parameter σ obtained theoretically, i.e. σ [flp-Pro-Gly]$_7 > \sigma$ [Pro-Hyp-Gly]$_7 > \sigma$ [Pro-Flp-Gly]$_7 > \sigma$ [Pro-Hyp-Gly]$_{10} > \sigma$ [Pro-Flp-Gly]$_{10}$ correlate distinctly with the thermal-stabilities of the collagen mimics. These theoretical observations are well reflected in the transition region (Figs. 2–6). The comparative degree of stability is further manifested by the shift in transition temperature T_f, i.e. T_f [flp-Pro-Gly]$_7 <$ T_f[Pro-Hyp-Gly]$_7 < T_f$ [Pro-Flp-Gly]$_7 < T_f$ [Pro-Hyp-Gly]$_{10} <$ T_f[Pro-Flp-Gly]$_{10}$ (refer to Table 1). The increase in the value of enthalpy change ΔH arrived at theoretically, may well be attributed to the increasing degree of stability of collagens and is primarily due to the fact, that smaller the value of σ, the larger will be the free energy penalty in creating the transition/boundary interface [27] (refer to Table 1). The introduction/substitution of the different atoms/groups like 4*R* hydroxyproline [Hyp] and 4*R* fluoroproline [Flp] 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 (refer to Table 1). The positional effects of γ substitution in addition to the electro-negativity [28] of the atoms/group and is further accentuated with the increase in chain length of collagen mimic. The increase in

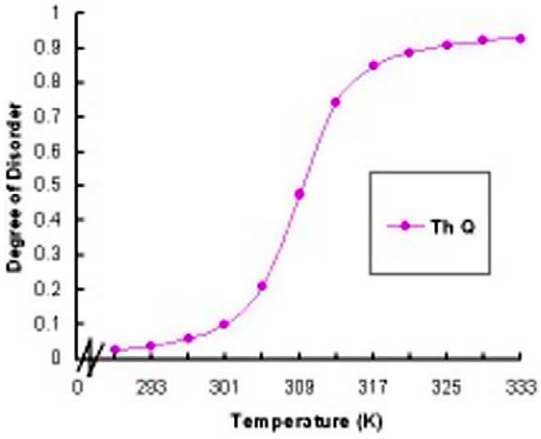

Fig. 3. Schematic representation of degree of disorder as a function of temperature in [Pro-Hyp-Gly]$_7$ (predictive values).

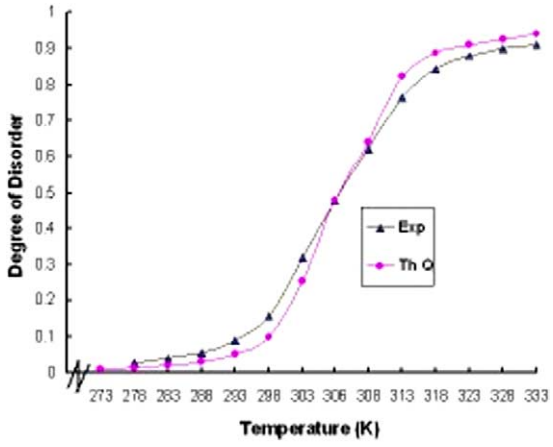

Fig. 2. Schematic representation of degree of disorder as a function of temperature in [flp-Pro-Gly]$_7$.

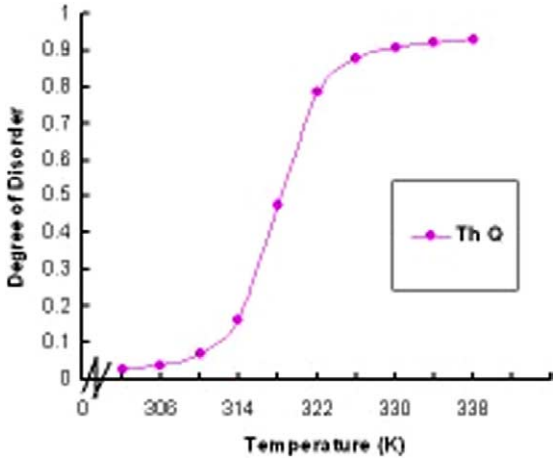

Fig. 4. Schematic representation of degree of disorder as a function of temperature in [Pro-Flp-Gly]$_7$ (predictive values).

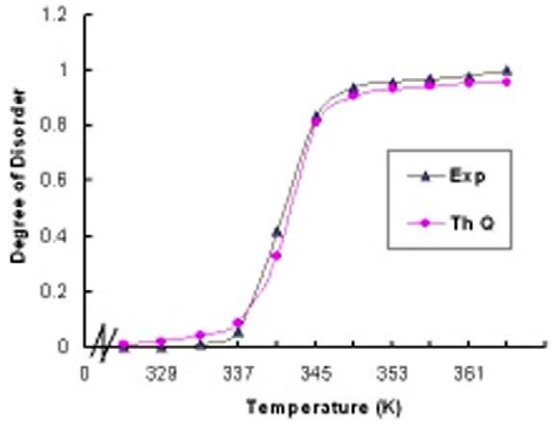

Fig. 5. Schematic representation of degree of disorder as a function of temperature in $[Pro\text{-}Hyp\text{-}Gly]_{10}$.

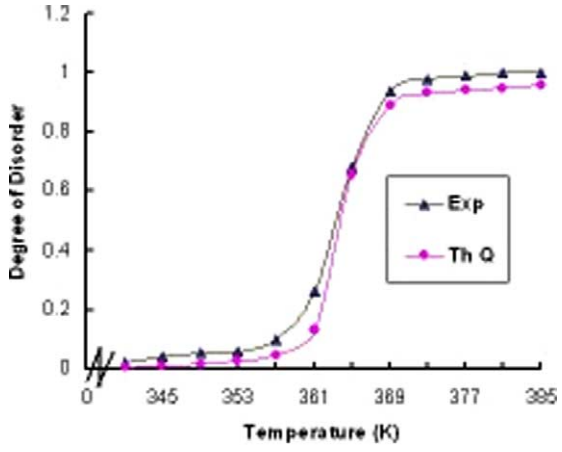

Fig. 6. Schematic representation of degree of disorder as a function of temperature in $[Pro\text{-}Flp\text{-}Gly]_{10}$.

transition temperature for a particular kind of substitution, either at first or second position of collagen mimics is also reflected by the decrease in σ values obtained theoretically, as the chain length increases.

In the collagen triple helix $(X\text{-}Y\text{-}Gly)_N$ all of the peptide bonds are in trans conformation, and the X and Y residues prefer C^{γ}-*endo* and C^{γ}-*exo* ring puckers, respectively. Since, 4(*R*) Hyp favours the C^{γ}-exo pucker, the Hyp in the Y position has a higher propensity to fold into a triple helix. Hyp in X position cannot adopt C^{γ}-endo pucker, as it destabilizes the triple helix. 4(*S*) Hyp although adopts the C^{γ}-endo pucker but with inappropriate ϕ torsion angle for Y position. In X position 4(*S*) Hyp is also likely to destabilize the triplex by a steric hinderance with Pro residue in Y position of another strand. Due to the gauche stabilization, the 4(*S*) fluoroproline (flp) residue would prefer to adopt a cis-exo conformation at the second position of mimic, but the resulting steric strain breaks the collagen molecule apart whereas the same cis-exo conformation results in a stable triple helix at the first position of collagen mimic. Hence, C^{γ} substituents at an appropriate position with right/favourable stereochemistry, i.e. 4(*R*)- and 4(*S*)- with [Hyp/Flp] residue can enhance the conformational stability and thereby pre-organizing the individual strands to resemble closely the strands in a triple helical state and could play an important role in protein engineering.

3. Conclusions

One of the merits of the present theoretical approach is that it leads to the arrival of values of nucleation parameter, which are consistent with the stabilization/destabilization of the ordered/disordered states subjected to various kinds of environmental conditions. The model successfully explains the effect due to the stereoelectronic/stereochemistry at C^{γ} position of proline in collagen mimics and hence the relative stability/instability of collagen mimics.

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