TEMPERATURE EFFECT ON COLLAGEN- LIKE STRUCTURE

Leopoldo Millan *) and Ezio Marchi**)

***)Techint, Bs As, Argentina**
****) Founder and First Director IMASL, UNSL – CONICET**
Emeritus Professor UNSL
(Ex) Superior Researcher CONICET
Correspondingauthor: emarchi1940@gmail.com

ABSTRACT:

In this paper we study a tropocollagen model in order to consider the temperature effect. Both situations with triple helix structure having one and two hydrogen bonds by each set of three amino acid are presented. Ising models are used for evaluating the statistical properties. Finally comparison with the experimental data is considered. The theoretical results agree very well with the experimental points.

1- INTRODUCTION

Collagen is present in ligaments, the matrix of bond and provides the intracellular binding substance in muscle and in other organs. Collagen can by solubilized, and with dilute acid converted into tropocollagen.

The tropocollagen is formed as a triple helix of length about $2{,}800\ \text{Å}$ and a diameter of about$15 - \text{Å}$. Each composing chain is a sequence of amino acid. One third of the total residues are glycine, about, one eight are proline and one in ten is hydroxy- proline. Each chain is binding with the other two by hydrogen bonds.

In the present paper we study the temperature effect in tropocollagen- like structures. We assume a simple version of the tropocollagen structure which is considered having only two components allow to form hydrogen bonds. These are hydroxyl- prolin and glycine.

It is very well know the structures of the triple helix of the tropocollagen. One model assumes that there are hydrogen bonds among hydroxyl- pralines and glycines respectively. On the other hand, a hypothetical model considers the no existence of glycine bonds among them. We study both situations.

We introduce a model consisting of molecules having two possible states. They represent the existence or not of hydrogen bond. Besides, the hydrophobic and electromagnetic interactions among molecules are also allowed. Principally, the cooperative effect is due to these interactions in the melting process.

We use an Ising model in order to evaluate the partition function from which macroscopie observable are derived.

2- COLLAGEN ISING MODEL

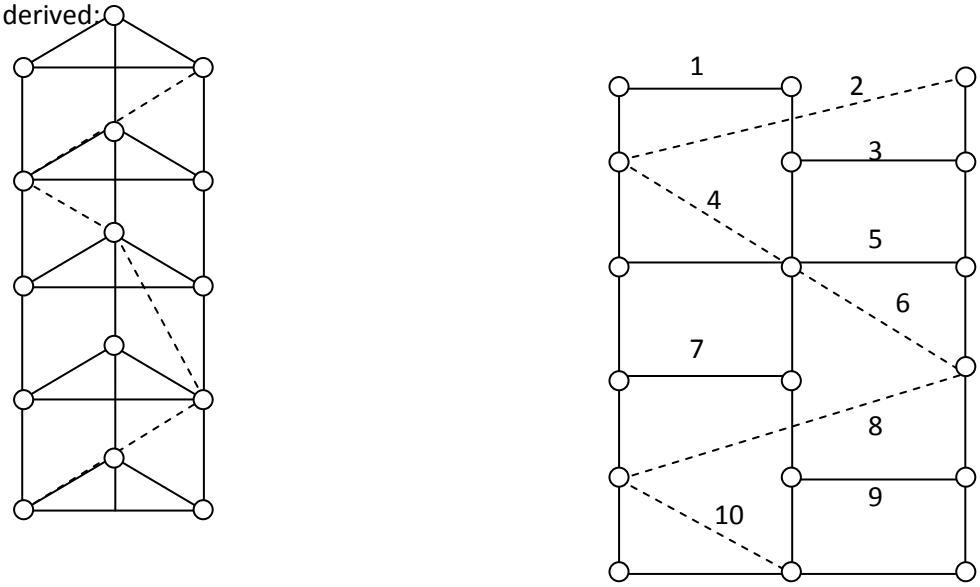
As we mentioned in the introduction, in this paragraph we are going to introduce and study some mathematical and physics facts of the theoretical model for the tropocollagen.

We are indeed interested in the bond breaking by temperature effect. Due to the fact that there is a correlation effect of interaction among certain bonds, there will appear a cooperative behavior in the bond breaking.

The two assuming visions about the collagen structures, that is to say either the existence or not of the hydrogen bond among glycine units, here appear together only parametrized by same suitable variable.

When these variables take extremes values, one obtains the two different theories.

First of all, we present in a the collagen structure in the first, from which some ideas can be derived:



On the left hand we have a collagen structure for its bond interaction. On the right, it is the same structure just planar. The bonds are of two types.

We remind that the vertical bonds are of covalent type and do not break with smooth changes of the temperature. Contrarly horizontal and slant bond are of hydrogen type and break by the effect of the temperature.

As it is mentioned by Poland and Scheraga[1]the helical configuration of most systematic ordered structure found in polymers is largely due to the non bonded interaction, that is to say the correlation or "steric" effect. Here we will study the collagen structure in the ising nearest-neighbor model, as described for example in Thompson [2].

In order to express the interactive energy among the molecule composing the collagen we indicate with $\varepsilon_1$and ε_2 the free energies of the hydrogen bonds between hydroxiproline and glycine respectively which are mark ked by o and in the figure 1. On the other hand, we introduce the correlation or "steric" energies which are $\bar{\alpha}, \bar{\beta}$and $\bar{\gamma}$. The first are represents the energy between ○ ○ and ○···○ ; the second are between ○···○ and ○···○ ; and finally between ○—○ and ○— ○ . All of them are considered as nearest- neighbors.

We use a set of parameter

$$\{\mu\} = \{\mu_1, \mu_2, \dots, \mu_N\}$$

in order to describe all the bond states, assuming that the collagen has N bonds or equivalently $\frac{3}{2}N$ principal molecules. For simplicity N is even. The possible values of μ_j are given as follows.

$$\mu_j = +1\ (j - bond\ intact) and$$
$$-1(j - bond\ broken)$$

where the position of the $j - th$ bond is show in the figure 1. As the reader may see the even $j's$ are those between glycine molecules and the odd ones between hydroxyproline molecules and the bond energies $\varepsilon_2$and ε_1 respectively. Thus, the total bond energy of the system is given by

$$E\big((\mu)\big) = -\bar{\varepsilon}_1 \sum_{j=1}^{N/2} \mu_{2j} - \bar{\varepsilon}_2 \sum_{j=1}^{N/2} \mu_{2j}$$

On the other hand, the total steric or correlation energy is expressed as following:

$$E_C\big((\mu)\big) = \frac{1}{Z} \sum_{j=1}^{N/2-1} \left(\mu_{2j}\mu_{2j+1} + \mu_{2j+1}\mu_{2j+2} + \mu_1\mu_2 + \bar{\beta} \sum_{j=1}^{N/2-1} \mu_{2j}\,\mu_{2j+2} + \bar{\gamma} \sum_{j=1}^{N/2-1} \mu_{2j-1}\,\mu_{2j+1} \right) \quad (2)$$

From here, the partition function of the system takes the form:

$$Z_N^{open} = \sum_{(\mu)} exp - \frac{1}{RT} E_B(\{\mu\} + E_C\{\mu\})$$

there$R$ is the gas constant and T the temperature.

3- **A)**<u>Partition Function Evaluation:</u>

Here we compute in some different cases the partition function. As a first step, we assume that the collage is a chain of molecules. This is a usual hypothesis for long polymer in the Ising context.

For example see Poland and Scheraga[1] and Wartell and Montrol. However, futher we will consider the “end effect” for a non- closed chain. We note that in both cases the transfer matrix is just the same. But in the latter case all the eigenvalues are taken into account.

Introducing the variables $\sigma_j = \big(\mu_{2j-1}\mu_{2j}\big)$ we then have the transfer matrix

$$L\big(\sigma_j, \sigma_{j+1}\big) = exp\varepsilon_1\mu_{2j-1} + \varepsilon_2\mu_{2j-2} - \alpha\,\mu_{2j}\,\mu_{2j+1} + \mu_{2j+1}\,\mu_{2j+2} - \beta\,\mu_{2j+1}\,\mu_{2j+2} - \gamma\,\mu_{2j-1}\,\mu_{2j+1}$$

were$\varepsilon_1 = \frac{\bar{\varepsilon}_i}{RT}$; $\alpha = \frac{\bar{\alpha}}{RT}$; $\beta = \frac{\bar{\beta}}{RT}$ and $\gamma = \frac{\bar{\gamma}}{RT}$

in the case with closed ends, the partition function appears to be given by

$$Z_N^{closed} = \sum_{\{\mu\}} exp - \frac{1}{RT} E_L(\{\mu\}) + E_C\left(\{\mu\} + \frac{\alpha}{2}\mu_N\mu_1\beta\mu_N\mu_2 + \bar{\gamma}\mu_{N-1}\mu_1\right) = \sum_{\substack{\{\sigma\} \\ j=1}}^{N/2} L(\sigma_j, \sigma_{j+1})$$

$$= TRACE\ L^{N/2} = \sum_{i=1}^{\lambda} \lambda_i^{N/2} \cong \lambda_{max}^{N/2}$$

We remind the line before the latter just expresses the matrix product on $N/2$ equal matrices L. The $\lambda's$ are the eigenvalues of the matrix L.

As a first case, we consider $\alpha = 0$, that is to say to correlation between hodrixiproline and glycine is negligible.

Therefore the maximum eigenvalue of $L$is obtained as

$$\lambda_{max} = e^{-(\beta+\gamma)}\{\cosh\varepsilon_1 + (\sinh^2\varepsilon_1 + e^4)\}^{1/2}\{\cosh\varepsilon_2 + (\sinh^2\varepsilon_2 + e^{4\beta})\}^{1/2}$$

As a second and interesting case, we have the situation when correlation between hidroxyproline and glycine is not negligible. However, in our analysis we need in such a case another restriction which is related with the existence of the hydrogen bond between glycine units. Here, we compute the characteristic polynomial corresponding to the transfer matrix L, resulting as:

$$P(\lambda) = \lambda^4 - p_1\lambda^3 + p_2\lambda^2 - p_3\lambda + p_4$$

$$= \lambda^4 - 2\,e^{-(\beta+\gamma)}[e^{-\alpha}\,cosh(\varepsilon_1+\varepsilon_2) + e^{\alpha}\,cos\,h(\varepsilon_1-\varepsilon_2)]^{\lambda^3}$$

$$+ \left[-4\,e^{-2\gamma}\,sinh\,2^{\beta}\,cosh\,2\varepsilon_1\right.$$

$$- 4\,e^{-2\beta}\,sinh\,2\gamma\,cosh\,2\varepsilon_2$$

$$\left.+ 4\,sinh^2\,\alpha\,cosh\,2(\beta+\gamma) - 4\,sinh\,2(\beta+\gamma)\,cosh^2\,\alpha\right]$$

$$- 8e^{-(\beta+\gamma)}(e^{\alpha}\,cosh(\varepsilon_1+\varepsilon_2) + e^{-\alpha}\,cosh(\varepsilon_1-\varepsilon_2)\,sinh\,2\beta\ \ h\,sin\,2\lambda$$

$$+ 4(cosh\,4\beta\,cosh\,4\gamma - cosh\,4\beta - cosh\,4\gamma + 1) = 0$$

The reader will realize that the task of compunting the root of the above characteristic polynomial is extremely complicated. Therefore we study a more simple but interesting case which appears when $p_1 = p_3$and $p_4 = 1$. These condition express that either

$\varepsilon_1 = 0\ or\ \varepsilon_2 = 0$.

This condition, physically is quite plausible since means in the case $\varepsilon_2 = 0$ that the hydrogen bond between glycine do not exist or is negligible.

Thus, the partition function in the "closed" case might be gotten. In the next section study the open case.

B)End effect:

Here we consider the collagen Ising model when no "closed" ends is assumed. In this case, that is to say with "open" ends and end effect will appear. However, we will show that the results are similar as the previous considered case since a small difference will be introduced as end effect.

We remember from (3) that

$$Z_N^{open} = \sum_{\sigma_1 \sigma_{N/2}} e^{-\alpha \mu_1 \mu_2 \frac{N}{L^2} 1} \tag{7}$$

$$\left(\sigma_1, \sigma_{N/2}\right) e^{\varepsilon_1 \mu_{N-1} + \varepsilon_2 \mu_N}$$

In order to reduce for computational purposes the last expression, let use consider the matrix L as $L = X \wedge X^{-1}$ where $\wedge$ is the respective diagonalized matrix of L and X matrix whose columns are the normalized eigenvectors respectively. Therefore

$$L(\sigma, \sigma^1) = \sum_{\tau} \sum_{\omega} X((\sigma, \tau) \lambda_2 \delta_{\tau\, \omega} X^{-1}, \omega, \sigma^1) = \sum_{\tau} X(\sigma, \tau)\, \lambda_\tau X^{-1}(\tau, \sigma^1) \tag{9}$$

The first equality is just the replacing of the diagonal matrix $\wedge$ where δ is Kronecker's delta. Since X is an orthonormal matrix, if holds $X^{-1}(\tau, \omega) = X(\omega, \tau)$ which implies that

$$L(\sigma, \sigma^1) = \sum_{\tau} \lambda_\tau\, X(\sigma, \tau) X(\sigma^1, \tau) \tag{10}$$

Similarly, it is easy to see that

$$L^s(\sigma, \sigma^1) = \sum_{\tau} \lambda_\tau X(\sigma, \tau) X(\sigma^1, \tau) \tag{11}$$

Now replacing (11) into (7), it appears that the partition function with end effect is

$$Z_N^{open} = \sum_{\tau} \lambda_\tau^{N-1}\, C_\tau \tag{12}$$

where

$$C_\tau = \sum_{\sigma 1 \quad \sigma} e^{-\alpha \mu_1 \mu_2}\, X(\sigma_1, \tau)\, \left(\sigma_{N/2}, \tau\right) e^{\varepsilon_1 \mu_{N-1} \varepsilon_2 \mu_N} \tag{13}$$

For large N, we can approximate

$$Z_N^{open} = \sum_{\tau} \lambda_{\tau}^{N/2-1} C_{\tau}$$

where is an index corresponding to the maximum eigenvalues. Because it is needed to compute

$$\log Z_N^{open} = \left(\frac{N}{2} - 1\right) log\, \lambda_{\bar{\tau}} + log\, \mathrm{C}_{\bar{\tau}} = \frac{N}{2} log\, \lambda_{\bar{\tau}} \tag{14}$$

for large N. From here we have that the end effect for very large N is negligible. This fact is satisfied in our case for the collagen.

Therefore, by such an argument, we do not go further into this matter.

4- MEDIUM NUMBER OF INTACT HYDROGEN BONDS

In this paragraph, we are going to consider and study the medium number or average of intact hydrogen bonds varying the temperature. This is in general obtained as

$$\theta_{\tau}(T) = \frac{1}{2\,N}\left(N + \frac{\partial}{\partial \varepsilon}\log \lambda_{max}^{N/2} + \frac{\partial}{\partial \varepsilon}\log \lambda_{max}^{N/2}\right) = \frac{M_I}{N}$$

were means the average.

Replacing λ_{max} in the closed case from the equation (5) into (15), we get

$$\theta_{\tau}(T) = \frac{1}{2}\left[1 + \frac{1}{2}\frac{\sinh \varepsilon_1}{(\sinh^2 \varepsilon_1 + e^{4\gamma})^{1/2}} + \frac{\sinh \varepsilon_2}{(\sinh^2 \varepsilon_2 + e^{4\beta})^{1/2}}\right] \tag{16}$$

On the other hand, in the case when$\varepsilon_2 = 0$, and in the reciprocal situation, it is possible to obtain$\theta_{\tau}(T)$.

It is interesting to see that in the closed chain case the condition of having a half of intact bonds, that is to say $\theta_1(T_m) = \frac{1}{2}$, where T_m is the respective temperature, it holds to a relation for correlation energies, namely

$$(\gamma - \beta) = \frac{1}{2}\log\left|\frac{\sinh \varepsilon_1}{\sinh \varepsilon_2}\right| \tag{17}$$

which can be easily derived from (16). In the case when $\beta = \gamma$ implies that $\varepsilon_1 = \varepsilon_2$. In general, because the hydroxyproline gives more stability to the collagen structure, $\varepsilon_1 > \varepsilon_2$, which means that $\gamma > \beta$. This happens at temperature T_m.

5- PARAMETERS EVALUATION

In this section we consider some aspect related with the evaluation of observable parameters. First of all, if the statistical weight contributing the partition function at temperature T is$exp\ \varepsilon(t)\ \mu_j$.

We here remind that this is the case of a unidimentional ring without correlation. Therefore at $T = T_m$ the statistical weight must be equal for balance reasons. Thus the free energy divided by $RT$is $\varepsilon(T_m) = 0$.

It is intuitively clear that the melting temperature and the free energies are different for the two bond types. This fact implies that the enthalphy and entropy are also different. then, we can write (18)

$$\varepsilon_1(T) = \frac{\Delta \mathrm{G}_1}{\mathrm{RT}} = \frac{\Delta \mathrm{H}_1 - \mathrm{T}\Delta \mathrm{S}_1}{\mathrm{RT}}$$

where$\Delta \mathrm{G}_1$is the free energy change at the bond breaking, similarly $\Delta \mathrm{H}_1$ the change of enthalpy and $\Delta \mathrm{S}_1$ the change of entropy. At the melting temperature T_m of the first type bonds, $\mu_1(T_m) = 0$.

from which: $\Delta \mathrm{H}_1 = T_{m_1}, \Delta \mathrm{S}_1$.

Therefore we obtain (19)

$$\varepsilon_1(T) = \frac{\Delta \mathrm{H}_1}{RT_{m_1}}\left(\frac{T_{m_1}}{T} - \hbar\right)$$

Similary, for the second type of bond.

Assuming that $T_{m_2} < T < T_{m_1}$ and near each other, then the equation (17) can be written at $T_m$as

$$e^{2(\gamma\beta)}\frac{\varepsilon_1(T_m)}{\varepsilon_2(T_m)} \cong \frac{\Delta 1}{\Delta \mathrm{H}_2}\Delta \tag{20}$$

where

$$\Delta\frac{T_{m_1} - T_M}{T_{m_2} - T_M}\frac{T_{m_2}}{T_{M_1}} \tag{21}$$

The previous equality is obtained by taking account (19).

From the equation (16) we may evaluate the derivative of the average of intact hydrogen bonds. After some manipulations and using (20) we get the following expression

$$\left(\frac{\partial\theta}{\partial}\right)_{T=T_M}\frac{\Delta \mathrm{H}_2}{2RT_M}e^{-2\beta}(1 + \Delta^{-1}) \tag{22}$$

from which we may relate β with the rate of change at $T = T_M$ of the average.

On the other hand, due to the fact that the entropy changes $\Delta \mathrm{S}_1$ and $\Delta \mathrm{S}_2$ might be considered equal, seince they take into consideration the degrees of freedom of broken

(23)

state; which are considered to be similar in the two types of molecules. With this, and considering (19) we obtain for the enthalpies

$$\frac{\Delta H_1}{T_{m_1}} = \frac{\Delta H_2}{T_{m_2}}$$

Using all the above relation (21), (22) and (23) we can evaluate $_1(T)$ in terms of the parameters T_{m_1}, T_{m_2}

$$\Delta H_2 \text{and} \left(\frac{\partial \theta}{\partial T}\right)_T = T_m$$

6- COMPARISON WITH EXPERIMENTAL DATA

From an experimental point of view the soluble collagen denaturation is considered as the change of intriensic viscosity as a temperature function. H. Boedtker and P. Doty in 1956 performed same experiments with soluble collagen in citrate at PH 3.7. their experimental results are used in this paper in order to compare them with the presented theory.

The enthalphy variation, following T. A. Orofino, A. Ciferri and J.J.

Hermans and D. Puett and L.U.Rajagh[6] is near $2kcal/mol$. The experimental value of $T_M \; is \; 29{,}2C$. with the use of (20), (22) and (23) and considering the equation (16) which gives the average of intact hydrogen bonds $\theta_I(T)$, taking into account proper values.

The denaturation curve very well adjusts the experimental points presented in 4).

In the second case when only when only the hydroxiproline can form hydrogen bonds, the theory agrees with experiments presented in

FINAL REMARKS

For both models we get from a practical point of view, identical denaturation curves. Therefore, one might not decide between them. The stability of both models might be explained on the following bases.

BIBLIOGRAPHY

[1] D. POLAND and H.A. SCHERAGA: Theory of Helix- Coil Transitions in Biopolymers Academic Press (1970)

[2] C.J. THOMPSON: Mathematical Statistical Mechanics. The Mc. Millan Co (1972)

[3] R.N. WARTELL and MONTROLL Advances in Chemical Physics 22 129 Wiley-Intersciences. New York (1972)

[4] H. BODTHER and P. DOTY J. Am. Chem. Soc. 75, 4267 (1956)

[5] T.A. OROFINO, A. CIFERRI and J.J. HERMANS, Biopolymers 5, 773 (1967)

[6] D. PUETT and L.V. RAJOH J. Macromol. Chem. A2, 111 (1968)

[7] VEIS A. The Macromolecular Chemistry of Gelatin. Academic Press. N. Yorks and London (1964) Cap. 1

ACKNOWLEDGEMENT

The authors would like to thank the Isaac Newton Institute for Mathematical Sciences for support and hospitality during the programme 'Discrete Analysis' when work on this paper was undertaken. This work was supported by

EPSRC Grant Number EP/K032208/1