

Order-order transition in a macromolecular system — poly-L-proline I and II

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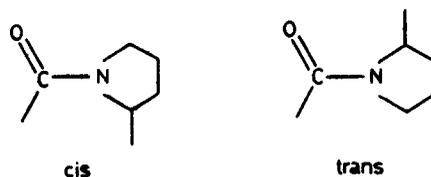
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Abstract. Ising model formalism developed for order-disorder transition in polypeptides has been modified for the study of order-order transition in a macromolecular system. A two-state theory is developed and applied to poly-L-proline I and II. Various transition curves for different chain lengths are obtained as a function of solvent composition. Transition curves and crossing point for a solvent system are in excellent agreement with the experimental data on poly-L-proline.

Keywords. Macromolecules; cooperativity; nucleation; transition matrix; boundary states; crossing point.

1. Introduction

The theory of order \leftrightarrow disorder transitions which has been applied to explain helix \leftrightarrow coil or crystalline \leftrightarrow amorphous transitions in a number of macromolecular systems (Mehrotra *et al* 1978, 1979; Agnihotri *et al*) is now modified to study the order-order transition in the case of poly-L-proline. Poly-L-proline exists, in solution, in two different forms designated as I and II (Katcholski *et al* 1965). In form I the peptide units are in the *cis* conformation (Traub and Schmueli 1963) which is a right-handed helix of *cis* imide groups with ten residues in three turns and an axial translation of 1.90 Å per residue. In form II the peptide units are in *trans* conformation (Cowan and McGavin 1955). The *trans* helix is lefthanded having three residues per turn and an axial translation of 3.12 Å per residue. The *trans* helix is thus relatively extended while the *cis* helix is compact (Rifkind and Applequist 1968).



Poly-L-proline exhibits a solvent induced transition from one form to the other. In contrast to the very fast α -helix \leftrightarrow coil transition (Schwarz 1964, 1965; Schwarz and Seelig 1968), the I \leftrightarrow II conversion is very slow. The rate is governed by the *cis-trans* isomerization of the peptide bond (Steinberg *et al* 1960) which depends on inter and intramolecular interactions. In compact *cis* form the attractive intramole-

cular interactions between hydrogens on C_α and C_β and the third oxygen dominate over the weak intermolecular (solvent-solute) interaction. The oxygen is at a van der Waals distance from the C_α and C_β hydrogens. In open *trans* form the intermolecular forces dominate the intramolecular forces which is evident from the fact that the *trans* form stabilizes in more strongly hydrogen bonding solvents.

It was discovered by Gornick *et al* (1964) that an equilibrium between the two forms exists in the solvent mixture *l*-propanol-acetic acid and that this equilibrium shifts from one form to the other within a narrow range of solvent composition. The equilibrium is cooperative, that is there is a tendency for peptide units in the same state to group together along the chain. An examination of molecular model suggests that the cooperative effect arises primarily from positive energy contributions as a result of electrostatic repulsions between charged oxygen atoms at the junctions between *cis* (I) and *trans* (II) peptide units in the molecule (Rifkind and Applequist 1968). The I-helix is stabilized by aliphatic alcohols and II-helix by trifluoroethanol, benzyl alcohol, water and organic acids. Infrared and other measurements show that only the functional group $C=O$ of poly-L-proline residues binds with alcohols by hydrogen bonds. Hence $I \leftrightarrow II$ conversion can be induced in other hydrogen bonding solvent systems also. Some of the difficulties and characteristics of order \leftrightarrow order transition are that:

(i) Unlike order \leftrightarrow disorder transition, both the states have difficulties to nucleate.

(ii) The molecular cause of cooperativity is quite different from that of order \leftrightarrow disorder transition as the two ordered states certainly do not differ much in entropy.

(iii) Solvent effect on $I \leftrightarrow II$ transition should be explained.

The two recent and significant theoretical approaches for order \leftrightarrow order transition are that of Schwarz (1967) and Applequist (1968). Both have used one-dimensional Ising model (Ising 1925) with nearest neighbour interactions to evaluate the partition function of the system, each residue or segment of which can exist in the two ordered states A and B. Schwarz developed the theory of Zimm and Bragg (1959) using two nucleation constants to take into account the four different nearest neighbour interactions AA, BB, AB, BA, residues but neglected the end effects in his transition matrix. He treated end effect as junction effect with two types of junctions AB or BA. Both agreed that the observed displacement of the transition curve for the short chain cannot be governed by the same effects which determine the sharpness of the transition for the long chains. Holzwarth (1969) and Holzwarth and Backman (1969) have shown that the observed cooperative nature of polyproline I-II transitions originates in part in the electrostatic interaction of the peptide group.

We report here, an extension of the one-dimensional Ising model to explain the order \leftrightarrow order transition through a two-parameter theory. The nucleation parameters for the growth of form I (II) in a sequence of II(I) are introduced. Also the end effects have been taken by weighting the contribution of first segment appropriately. Using the matrix method (Kramers and Wannier 1960) the partition function for a chain of length N is obtained and hence Q , the fraction in the form I. The theoretical transition curves obtained for various values of N and nucleation parameters, are then adopted for the solvent systems, trifluoroethanol-*n*-butanol and benzyl alcohol-*n*-butanol and compared with the transition curves of Ganser *et al* (1970).

2. Theory

The segments in the form I and II are represented by h_1 and h_2 and k_1 and k_2 the boundary states, being first of a sequence of segments in states I and II, respectively. The growth parameters are s_1 and s_2 and σ_1 and σ_2 are the nucleation parameters for segments in state I and II respectively. The transition matrix for order \leftrightarrow order transition is then as follows:

$$M = \begin{matrix} & h_1 & k_1 & k_2 & h_2 \\ \begin{matrix} h_1 \\ k_1 \\ k_2 \\ h_2 \end{matrix} & \begin{pmatrix} s_1 & 0 & \sigma_2 s_2 & 0 \\ s_1 & 0 & 0 & 0 \\ 0 & 0 & 0 & s_2 \\ 0 & \sigma_1 s_1 & 0 & s_2 \end{pmatrix} \end{matrix} \quad (1)$$

The four eigenroots of M are given by the secular equation:

$$|M - \lambda I| = 0$$

which becomes

$$\lambda^2(\lambda - s_1)(\lambda - s_2) - \sigma_1 \sigma_2 s_1^2 s_2^2 = 0. \quad (2)$$

These eigenroots depend only on the product $\sigma_1 \sigma_2 (= \sigma$ say). If $\sigma_1 \sigma_2 = 0$ the eigenroots are $s_1, s_2, 0, 0$ and if $\sigma_1 \sigma_2 \ll 1$ the two smaller eigenroots are of the order of $(\sigma_1 \sigma_2)^{1/2}$ and the larger eigenroots are of the order of s_1 and s_2 that is unity in the transition range. Thus the contribution of smaller eigenroots to partition function is negligible and the main contribution comes from the larger eigenroots which are denoted by λ_1 and λ_2 . These eigenroots can be obtained on iteration and are given by the following relations:

$$\lambda = s_1 + \frac{\sigma_1 \sigma_2 s_1^2 s_2^2}{\lambda^2 (\lambda - s_2)}, \quad (3a)$$

or

$$\lambda = s_2 + \frac{\sigma_1 \sigma_2 s_1^2 s_2^2}{\lambda^2 (\lambda - s_1)}. \quad (3b)$$

To calculate partition function for a chain of N segments we need to know M^N . The diagonalising matrices for M are:

$$T = \begin{bmatrix} \frac{\lambda_1}{s_1} & \frac{\lambda_2}{s_1} & \frac{\lambda_3}{s_1} & \frac{\lambda_4}{s_1} \\ 1 & 1 & 1 & 1 \\ \frac{\lambda_1(\lambda_1 - s_1)}{\sigma_2 s_1 s_2} & \frac{\lambda_2(\lambda_2 - s_1)}{\sigma_2 s_1 s_2} & \frac{\lambda_3(\lambda_3 - s_1)}{\sigma_2 s_1 s_2} & \frac{\lambda_4(\lambda_4 - s_1)}{\sigma_2 s_1 s_2} \\ \frac{\lambda_1^2(\lambda_1 - s_1)}{\sigma_2 s_1 s_2^2} & \frac{\lambda_2^2(\lambda_2 - s_1)}{\sigma_2 s_1 s_2^2} & \frac{\lambda_3^2(\lambda_3 - s_1)}{\sigma_2 s_1 s_2^2} & \frac{\lambda_4^2(\lambda_4 - s_1)}{\sigma_2 s_1 s_2^2} \end{bmatrix}, \quad (4a)$$

$$T^{-1} = \begin{bmatrix} \frac{C_1 s_1}{\lambda_1 - s_1} & C_1 & \frac{C_1 \sigma_2 s_1 s_2}{\lambda_1 (\lambda_1 - s_1)} & \frac{C_1 \lambda_1}{\sigma_1 s_1} \\ \frac{C_2 s_1}{\lambda_2 - s_1} & C_2 & \frac{C_2 \sigma_2 s_1 s_2}{\lambda_2 (\lambda_2 - s_1)} & \frac{C_2 \lambda_2}{\sigma_1 s_1} \\ \frac{C_3 s_1}{\lambda_3 - s_1} & C_3 & \frac{C_3 \sigma_2 s_1 s_2}{\lambda_3 (\lambda_3 - s_1)} & \frac{C_3 \lambda_3}{\sigma_1 s_1} \\ \frac{C_4 s_1}{\lambda_4 - s_1} & C_4 & \frac{C_4 \sigma_2 s_1 s_2}{\lambda_4 (\lambda_4 - s_1)} & \frac{C_4 \lambda_4}{\sigma_1 s_1} \end{bmatrix} \quad (4b)$$

The normalization constants C_1, C_2, C_3 and C_4 are given by relation:

$$C_i = \frac{(\lambda_i - s_1)(\lambda_i - s_2)}{4\lambda_i^2 - 3\lambda_i(s_1 + s_2) + 2s_1 s_2} \quad \text{where } i = 1, 2, 3, 4. \quad (5)$$

The vector U gives the contribution of the first segment (left end)

$$U = (\sigma' s_1, 0, 0, \sigma'' s_2), \quad (6)$$

with σ' and σ'' as the parameters giving the interaction of end segments with the solvent when they are respectively in states h_1 and h_2 . In all our calculations it is their ratio $x = \sigma''/\sigma'$ which will be meaningful.

The partition function of the chain is then given by

$$Z = U T T^{-1} M^{N-1} T T^{-1} V, \quad (7)$$

where the column vector $V = \begin{pmatrix} 1 \\ 1 \\ 1 \\ 1 \end{pmatrix}$,

assuming equal probabilities for all the four states k_1, h_1, k_2 and h_2 on the right end. On putting values of U, T, T^{-1}, M and V in (7) we have

$$\begin{aligned} Z &= \sigma' \sum_{i=1}^4 \frac{\lambda_i^N [\lambda_i^2 (1+x) - \lambda_i (s_2 + s_1 x) + s_1 s_2 (\sigma_1 x + \sigma_2)]}{4\lambda_i^2 - 3\lambda_i (s_1 + s_2) + 2s_1 s_2} \\ &= \sigma' \sum_{i=1}^4 A_i \lambda_i^N, \end{aligned} \quad (8)$$

$$\text{with } A_i = \frac{\lambda_i^2 (1+x) - \lambda_i (s_2 + s_1 x) + s_1 s_2 (\sigma_1 x + \sigma_2)}{4\lambda_i^2 - 3\lambda_i (s_1 + s_2) + 2s_1 s_2}. \quad (9)$$

Now
$$\frac{\partial Z}{\partial s_1} = \sigma' \sum_{i=1}^2 N \lambda_i^N \left(\frac{1}{N} \frac{\partial A_i}{\partial s_1} + \frac{A_i}{\lambda_i} \frac{\partial \lambda_i}{\partial s_1} \right), \quad (10)$$

neglecting the sum over $i = 3$ and 4 since the eigenroots λ_3 and λ_4 are very small as compared with others. Q_I the fraction of the states in the form I can now be calculated from:

$$Q_I = \frac{1}{N} \frac{\partial \ln Z}{\partial \ln s_1} = \frac{1}{N} \frac{s_1}{Z} \frac{\partial Z}{\partial s_1} \\ \cong \frac{\frac{s_1}{\lambda_1} \frac{\partial \lambda_1}{\partial s_1} + \frac{s_1}{\lambda_2} \frac{\partial \lambda_2}{\partial s_1} \cdot B + \frac{1}{N} \left[\frac{s_1}{A_1} \frac{\partial A_1}{\partial s_1} + \frac{s_1}{A_2} \frac{\partial A_2}{\partial s_1} \cdot B \right]}{1 + B}, \quad (11)$$

where $B = \frac{A_2}{A_1} \left(\frac{\lambda_2}{\lambda_1} \right)^N$.

Obviously $Q_{II} = 1 - Q_I$. (12)

To evaluate $\partial A_1/\partial S_1$ and $\partial A_2/\partial S_1$, differentiating A_i (equation (9)) with respect to s_1 gives

$$\frac{\partial A_i}{\partial s_1} = \frac{k}{s_1 b_i^3} \left[(s_2 - s_1) y \lambda_i^3 + 2 \sigma_1 \sigma_2 k \lambda_i \{s_1 (3 - x) + s_2 (1 - 3x)\} \right. \\ \left. + \frac{\sigma_1 \sigma_2 k^2}{\lambda_i} \{s_1 (-2x + 3y) + s_2 (2 - 3y) - 8 \sigma_1 \sigma_2 k^2 (x - 1)\} \right] \\ = \frac{k}{s_1 b_i^3} \left[(s_2 - s_1) y \lambda_i^3 + 2 \sigma k \lambda_i \{s_1 (3 - x) + s_2 (1 - 3x)\} \right. \\ \left. + \frac{\sigma k^2}{\lambda_i} \{s_1 (-2x + 3y) + s_2 (2 - 3y) - 8 \sigma k^2 (x - 1)\} \right], \quad (13)$$

where $b_i = 4\lambda_i^2 - 3\lambda_i (s_1 + s_2) + 2k$, ($i = 1, 2$),

$y = (\sigma_1 x + \sigma_2)$,

and $k = s_1 s_2$,

Similarly $\partial \lambda_1/\partial s_1$ and $\partial \lambda_2/\partial s_2$ from (2) are given by:

$$\frac{\partial \lambda_i}{\partial s_1} = \frac{\lambda_i (\lambda_i - s_2) (2\lambda_i - s_1)}{s_1 b_i}. \quad (14)$$

The equilibrium constant s for transition is

$$s = \frac{s_1}{s_2} = \frac{s_1^2}{k}$$

Since every turn of helix I which uncoils itself goes into helix II and vice versa, one could, therefore, assume to a first order approximation that $k = 1$ or $s_1 s_2 = 1$. In a phase transition s is related to the energy of transition. Different values of s will, therefore, mean the different values of energies. From the experimental data these can be related to the solvent composition. From (12), (13) and (14) Q_1 for very large N becomes:

$$Q_1 = \frac{\frac{s_1}{\lambda_1} \frac{\partial \lambda_1}{\partial s_1} + \frac{s_2}{\lambda_2} \frac{\partial \lambda_2}{\partial s_1} \cdot B}{1 + B} \quad (15)$$

Since B also tends to zero for N infinitely large, Q_1 is given by:

$$Q_1 = \frac{s_1}{\lambda_1} \cdot \frac{\partial \lambda_1}{\partial s_1}, \quad \lambda_1 \text{ denoting the largest eigenroot.}$$

It is noticed that $\partial A_1 / \partial s_1$ and $\partial A_2 / \partial s_1$ change sign in the neighbourhood of $s \simeq 1$ i.e. probabilities of occupation of states I and II are extremum in this region. Except for small values of N , the crossing point occurs when Q_1 becomes independent of N .

Thus

$$\frac{s_1}{A_1} \cdot \frac{\partial A_1}{\partial s_1} + \frac{s_2}{A_2} \frac{\partial A_2}{\partial s_1} \cdot B = 0$$

The crossing point also depends on the values of the parameters k and x .

3. Results and discussion

Three sets of theoretical transition curves (Q_1 versus solvent composition) for different chain lengths obtained from equations (11) to (15) and experimental points are shown in figure 1 ($Q_1 \equiv \theta_1$). Various transition parameters which provide best fit to the experimental data of Ganser *et al* (1970) are given in table 1.

As discussed earlier $k (= s_1 s_2)$ has been chosen to be equal to unity. The parameter $\sigma (= \sigma_1 \sigma_2)$ is taken to be constant assuming the solvent effect on growth only and not on the nucleation of the two forms of poly-L-proline within the chain i.e.

Table 1. Transition parameters.

Set	Solvent system	k	σ	σ_1	σ_2	x	Crossing point at Q_1
I	TFE- n -butanol at 25°C	1	5×10^{-5}	7.07×10^{-3}	7.07×10^{-3}	0.3	0.77
II	TFE- n -butanol at 70°C	1	5×10^{-5}	4×10^{-3}	1.25×10^{-3}	1.5	0.43
III	Benzyl alcohol- n -butanol at 70°C	1	5×10^{-5}	5×10^{-3}	1×10^{-3}	15	0.09

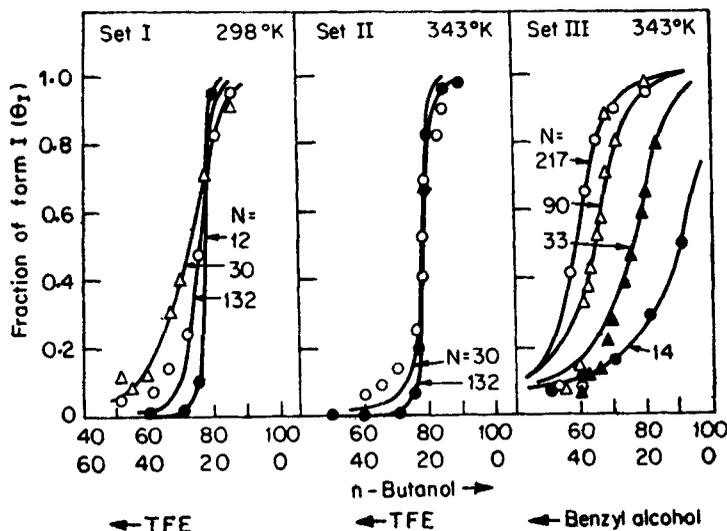


Figure 1. Equilibrium transition curves for various solvent systems and chain lengths (N).

cooperativity is not changed by the binding of the solvent. The parameter σ is adjusted and is found to be about 5×10^{-5} for the best fit of the transition curves for $N \rightarrow \infty$ where the end effect can be neglected and the fraction of form I is approximated to $Q_I = (s_1/\lambda_1) (\partial\lambda_1/\partial s_1)$. This is based on the fact that the contributions to the partition function from other eigenroots are negligible as compared to those from the largest eigenroot λ_1 . The largest eigenroot λ_1 is given by the relation (2) wherein only parameter σ is involved. Having fixed the value of σ as stated above, the slope of the transition curves is altered by varying σ_1 . The parameter σ_1 is adjusted for the transition curves corresponding to different N values. Since the slope of the transition curves depend on σ_1 , it is adjusted for the best fit with experimental data. These values for the three sets are given in table 1. The variation of σ_1 and σ_2 means the variation of probabilities of nucleation of form I in a sequence of segments II and the nucleation of form II in a sequence of segments I respectively. The choice of end effect parameter x depends on the adjustment of crossing point.

Order \leftrightarrow order transition in poly-L-proline is affected by temperature and solvent composition. Form I is stabilized in *n*-butanol and form II is stabilized in TFE and benzyl alcohol. Benzyl alcohol is a strong hydrogen bonding solvent as compared to TFE.

In the first case (set I) poly-L-proline is at lower temperature (25°C) and is in weak hydrogen bonding solvent system TFE. Here $\sigma_1 = \sigma_2$, assuming that nucleations of form I and form II are equally probable within the chain. But the end effect parameter x is less than one ($x = 0.3$) that is $\sigma' > \sigma''$ which implies that at the end nucleation of form I is more probable than the nucleation of form II because intramolecular interactions dominate over intermolecular interactions. In the second case (set II) poly-L-proline is in the same solvent system but at higher temperature (70°C) when compared with the previous case; in this case the effect on order \leftrightarrow order transition can be attributed to the temperature only. Here $\sigma_1 > \sigma_2$ which implies that within the chain, nucleation of form I is more probable than the nucleation of form II because intramolecular interactions dominate over intermolecular inter-

actions. But the end effect parameter is more than unity ($x = 1.5$) i.e. $\sigma'' > \sigma'$ which implies that at the ends nucleation of form II is more probable than the nucleation of form I because intermolecular interactions dominate over intramolecular interactions at end. Thus the rise in temperature provides sufficient thermal energy to break weak intramolecular hydrogen bonds. In the third case (set III) poly-L-proline is at a higher temperature (70°C) dissolved in a strong hydrogen bonding solvent—benzyl alcohol. Now σ_2 is greater than σ_1 which implies that the nucleation of form II is more probable than the nucleation of form I while in the second case the position is reversed. This is indicative of the fact that strong hydrogen bonding solvents retain form II even within the chain. The end effect parameter is further increased ($x = 15$) making nucleation of form II still more probable. In conclusion, it may be added that one of the merits of the present approach consists in explicitly arriving at numerical values of the initiation parameters σ_1 and σ_2 (table 1) and interpreting them consistently with the available experimental data. Thus the effect on order \leftrightarrow order transition of poly-L-proline can be attributed to both temperature and solvent composition. The intermolecular interactions dominate over intramolecular interactions both within the chain and at the end.

References

- Agnihotri R C, Mehrotra C and Gupta V D 1981 *J. Phys. C* (communicated)
Applequist J 1968 *Biopolymers* **6** 117
Cowan R M and McGavin S 1955 *J. Am. Chem. Soc.* **176** 1062
Ganser V, Engel J, Winklmair D and Krause G 1970 *Biopolymers* **9** 329
Gornick F, Mandelkern L, Diorio A F and Roberts D S 1964 *J. Am. Chem. Soc.* **86** 2549
Holzwarth G 1969 *Macromolecules* **3** 245
Holzwarth G and Backman K 1969 *Biochemistry* **8** 883
Ising E 1925 *Z. Phys.* **31** 253
Katcholski E, Berger A and Kurtz J 1965 *Aspects of protein structure*, (ed.) G N Ramachandran (New York: Academic Press) p. 205
Kramers H A and Wannier G H 1941 *Phys. Rev.* **60** 252
Mehrotra C, Agnihotri R C and Gupta V D 1978 *Indian J. Pure Appl. Phys.* **16** 29
Mehrotra C, Agnihotri R C and Gupta V D 1979 *Natl. Acad. Sci. Lett.* **2** 75
Rifkind J M and Applequist J 1968 *J. Am. Chem. Soc.* **90** 3650
Schwarz G 1964 *Ber. Bunsenges Physik Chem.* **68** 843
Schwarz G 1965 *J. Mol. Biol.* **11** 64
Schwarz G 1967 *Biopolymers* **5** 321
Schwarz G and Seelig J 1968 *Biopolymers* **6** 1263
Steinberg I Z, Harrington W F, Berger A, Sela M and Katcholski E 1960 *J. Am. Chem. Soc.* **82** 5263
Traub W and Schmueli U 1963 *Nature (London)* **198** 1165
Zimm B H and Bragg J K 1959 *J. Chem. Phys.* **31** 526