Coil-helix transition in poly(L-glutamic acid): Evidence for a 3-state non-cooperative process

G. Zalczer
Service de Physique de l’Etat Condensé, CEA Saclay, 91191 Gif-sur-Yvette cedex, France

Abstract. A careful analysis of measurements of circular dichroism of poly(L-glutamic acid) (PGA) shows that the data can be very accurately described by introducing a third state for the PGA configuration, in addition to the helix and coil ones, and considering a simple equilibrium between these three states, without cooperativity. The third state is more conspicuous when high molecular weight polyethylene glycol (PEG) is added. Excluded-volume effects shown by differences in the presence of short and long PEG chains suggest a direct interaction of PEG and PGA rather than an osmotic effect.

1 Introduction
Proteins are essential building blocks of life. They exhibit multiple properties due to the interplay between their four structural levels: primary (aminoacids sequence), secondary (alpha helices, beta sheets, etc.), tertiary (arrangements of secondary structures in space) and quaternary (self-assembly of supramolecular structures). The primary structure is fixed by covalent bonds and is therefore very stable. The other levels of order are governed by much weaker interactions (hydrogen bonds, dipolar interactions, etc.) and are therefore sensitive to changes in the environment such as temperature, pH, etc. Understanding the formation of these structures is an important step in the understanding of their overall properties. Moreover it is strongly suspected that the misfolding of some proteins is responsible for serious diseases such as prion-related diseases or Alzheimer’s disease [1]. We focus here on the formation of alpha helices from random coils in a much simplified system: poly(L-glutamic acid) (PGA). This system has been previously studied due to its reported particularity of presenting only these two states. The helical parts are optically active and their population can be easily and accurately measured by circular dichroism.

This transition is usually analysed in terms of a theory due to Zimm and Bragg [2,3] (or variants thereof [4-6]). In this theory an elementary segment of the chain can exist in two states: either as a disordered chain or as a loop. A free-energy difference between these states comprises energy and entropy terms
\[ \Delta F = \Delta E - T \Delta S, \]
where \( \Delta E \) and \( \Delta S \) can be considered as independent of temperature in a first approach. (A more complete description [7] introduced a linear temperature variation in \( \Delta E \) and a logarithmic one in \( \Delta S \). The non-linearity in the log function in the range 280–370 K is too small to be seen so that eq. (1) applies with a different meaning of \( E \) and \( S \).) We choose the energy unit such that \( k_B = 1 \). In the absence of other interactions, the relative ratio of states would be given by a Boltzmann factor \( \exp(-\Delta F/T) \). In addition, Zimm and Bragg introduced an interaction between neighbouring elements which facilitates the formation of a new loop beside an existing one and therefore the transition of the whole chain. The problem is therefore similar to the 1D Ising model and can be solved.

Many sets of data have been analysed using this model [6,8-10] and significant cooperation parameters determined even though a glance at the presented figures shows a rather poor fit to the data. A new set of circular dichroism measurements using solutions with added polyethylene glycol (PEG) and at \( \text{pH}=3.75 \) have been performed using state-of-the-art equipment [11] and will be the basis of our analysis. The results are plotted in fig. 1.

Without PEG, the transition occurs at low temperature and only a part of it can be studied. The addition of PEG shifts the transition to higher temperatures but modifies the system. The figure suggests however that these modifications do not qualitatively change the basic mechanism.

2 Critical plot of the data
A clearer picture can be obtained by plotting the data in a different way. Indeed eq. (1) can be rewritten as
\[ \log\left[\frac{x}{(1-x)}\right] = \frac{\Delta E}{T} - \Delta S, \]

\( a \) e-mail: Gilbert.Zalczer@cea.fr
where $x$ is the fraction of helices. In the absence of cooperativity a plot of $\log(x/(1-x))$ vs. $1/T$ would be a straight line while strong cooperativity would lead to a sigmoidal shape.

Computing the helical fraction $x$ is, however, not straightforward because it requires the knowledge of the asymptotic values reached by the curves of fig. 1 at high and low temperature, $hTlv$ and $lTlv$ corresponding to fractions of helices of 0% and 100%. These are the optical properties of given molecular conformations and should not vary appreciably with temperature. The high-temperature limit can be safely assessed for the data with 0% and 5% PEG, but the difference between these two values indicates some lack of reproducibility between runs. A value of 37.4° has been published [12] for the helix maximum contribution, allowing for an estimate of $lTlv$. We allowed for a variation of a few degrees of each of these values to get the most consistent pattern. The results are shown in fig. 2.

We notice first that all curves of fig. 2 have a similar shape: two linear parts at low and high temperature matched by a smooth crossover.

### 3 The 3-state model

Obviously the data do not look like any of the shapes predicted by the theory of Zimm and Bragg. However, the presence of two linear parts in the graph hints that two transition free energies and therefore three thermodynamic states are involved. The fraction of helices, in the absence of cooperativity, is easily computed from Boltzmann factors

$$\frac{x}{1-x} = \frac{\exp(-\Delta F_1/T)}{1 + \exp(-\Delta F_2/T)},$$

or

$$\frac{x}{1-x} = \exp(-\Delta E_1/T - \Delta S_1)\left(1 + \exp(-\Delta E_2/T - \Delta S_2)\right),$$

taking the coil state to be the reference state (we assume that only one state is optically active).

The raw data have been fitted to the formula

$$hTlv + (lTlv - hTlv)x,$$

where $hTlv$($lTlv$) is the high (low) temperature limit value and $x$ is obtained from eq. (4). Each curve can be fitted very accurately using 6 independent parameters ($\Delta E_1$, $\Delta S_1$, $\Delta E_2$, $\Delta S_2$, $hTlv$, $lTlv$). The values found for the limiting values and the energies are reasonable. However, many of these values cannot be considered as precisely determined. Indeed the number of data points in the high helicity side of the PEG-poor samples and in the low helicity side of the PEG-rich samples is not sufficient. Moreover a slight change in a limit value leads to a change in the corresponding energy without significantly altering the quality of the fit. Among the many possible ways of constraining the fit, we choose to explore a possibility suggested by the figures, namely constant energies (slopes) for all concentrations. The trial energies are taken from the best fit of the “best balanced” curve at 10% PEG. They are 10400 K and 7780 K or 21 kCal M$^{-1}$ and 15.5 kCal M$^{-1}$.

The fit of the experimental data with this formula can be considered as quite satisfactory for all the samples including PEG as can be seen in fig. 1–3 (which also justifies our assumption that only one state is optically active). The data without PEG, however, did exhibit a systematic deviation and a different set of values of energies had to be found. The values are 13500 K and 8870 K or 27 kCal M$^{-1}$ and 17.8 kCal M$^{-1}$. This fit is the one plotted in fig. 1 and fig. 2. This “constant energy” constraint is only heuristic,
and fits assuming constant entropies and adjustable energies are as good.

For comparison, we also tried to fit the data (namely the 10% PEG curve) with the Zimm-Bragg formula. Using blindly the fitting program led to unrealistic results such as a tiltv of about $-35^\circ$. We therefore constrained the asymptotic values $hltv$ and $ltlv$ between $-4^\circ$ and $-8^\circ$, and $-39^\circ$ and $-45^\circ$, respectively. The best fit shows a strong systematic deviation reported in fig. 3. Moreover the cooperativity energy found has the opposite sign of that expected, corresponding to an antagonism rather than a cooperativity.

4 Effect of PEG chain length

The results of a similar study were published a few years ago [10]. The main difference is that its authors used short PEG chains while we [11] used long PEG chains. The data (picked from the published figure) can be plotted using the same process as above (fig. 4). Consideration of fig. 4 reveals similarities and differences. First, the same pattern of two transitions matched by a crossover is apparent, but the crossover occurs at lower temperatures preventing us from performing any quantitative analysis for this state. The energy difference between the coil and helix states (slope of the data at small $1/T$) is equal to that observed for long chains. A striking feature is that the addition of some short PEG always has a roughly constant effect, while the shift of the data due to adding long PEG clearly levels off. To illustrate this point, we have plotted the temperature $T_1 = E_1/S_1$ vs. the fraction of PEG for all the curves (fig. 5). The data cannot be compared one to one because of the different pH values but the trend of the curves seems significant. The idea is that a peptide can interact with more and more small molecules when the concentration of these molecules is increased, while the excluded-volume effects prevent this for macromolecules.

This shift therefore probably has an entropic origin. A mechanism related to osmotic stress, as suggested by Stanley and Strey [10] should depend on the concentration of PEG but not on the length of the chains.

5 Final remarks

The presence of a third state between coil and helix should not be too surprising. Indeed, the amino acids involved are very prone to interacting with each other leading to different structures. A hairpin structure, precursor of a beta sheet, could be a likely hypothesis. An aggregated state would have a much lower entropy and could therefore occur at the lowest temperatures but its occurrence at intermediate temperature is not likely. A similar study with
different lengths of another peptide molecules (AEAAKA) has been published [8,9]. Our analysis of the data from the published figures shows that the third state is not visible for the shortest chains (up to 26 residues) but seems to appear for the longer ones (32 or more residues). This clearly favors a third state induced by a self-interaction of the chains. The characterisation of the third state by neutron or X-ray scattering can be envisioned but the simultaneous presence of several conformations would make the unraveling of the spectra difficult. An X-ray study by Muroga et al. [13] concluded that the observed data did not fit with what was expected for a coil in the region where a coil was expected. Indeed the observed spectra decrease more slowly than expected at large wave vectors, indicating a more compact state of the scattering objects. A study of the dynamics of the helix-coil transition for PGA also concluded that this process was not a two-state one [14].

6 Conclusion

The first conclusion of this study is that the sharpening of the transition due to first-neighbour interaction, as predicted by Zimm and Bragg [2], is not observed. Instead the consideration of three states in thermodynamical equilibrium allows to describe accurately the data. The nature of the third state, which may have significant importance in health-related problems, remains to be investigated as does the exact interaction of PGA with PEG. The evidence of excluded-volume effects suggests a direct interaction. Circular dichroism measurements for PEG concentrations between 0% and 5% could be useful for this purpose.

References

1. F. Chiti, C.M. Dobson, Annu. Rev. Biochem. 75, 333 (2006).
2. B.H. Zimm, J.K. Bragg, J. Chem. Phys. 31, 526 (1959).
3. A. Grosberg, A. Khokhlov, Statistical Physics of Macromolecules (AIP Press, New York, 1994).
4. S. Lifson, A.J. Roig, Chem. Phys. 34, 1963 (1961).
5. O. Farago, P. Pincus, Eur. Phys. J. E 8, 393 (2002).
6. K. Ghosh, K.A.J. Dill, Am. Chem. Soc. 131, 2306 (2009).
7. V. Muñoz, L.J. Serrano, Mol. Biol. 245, 297 (1995).
8. J.M. Scholtz, H. Qian, E.J. York, J.M. Stewart, R.L. Baldwin, Biopolymers 31, 1463 (1991).
9. R.L. Baldwin, Biophys. Chem. 55, 127 (1995).
10. C.B. Stanley, H.H. Strey, Biophys. J. 94, 4427 (2008).
11. A. Koutsouhbas, D. Lairez, S. Combet, G. Fadda, S. Longeville, G. Zalczer, J. Chem. Phys. 136, 215101 (2012).
12. J. Su, R. Hodges, C. Kay, Biochemistry 33, 15501 (1994).
13. Y. Muroga, H. Tagawa, Y. Hiragi, T. Ueki, M. Kataoka, Y. Izumi, Y. Anemiya, Macromolecules 21, 2756 (1988).
14. S. Sharma, Thesis, University of Liverpool, UK (2006).