THE STABILITY OF THE HELICAL CONFORMATION OF RANDOM L-GLUTAMIC ACID–2-NITROBENZYL-L-GLUTAMATE COPOLYMERS IN AQUEOUS SOLUTION

Jacky ESTEVEZ and Marie-H. LOUCHEUX-LEFEBVRE*

Laboratoire de Chimie Macromoléculaire, Université des Sciences et Techniques de Lille, B.P. 36, 59650 Villeneuve d.Ascq France

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1. Introduction

It is well known that hydrophobic forces such as the interactions of aromatic side chains with each other and with the aqueous solvent play an important role in stabilization of native structures in proteins [1]. Because the analogy between the physical-chemical properties of synthetic poly-α-amino acids and proteins, the factors responsible for conformational stability of synthetic polypeptides were investigated from both an experimental and a theoretical point of view. Since an important study on secondary structure has been done with poly-L-glutamic acid, we have used this polymer as a model for examining the effect of the incorporation of an amino acid with a hydrophobic side chain. In the present work, the helical stability of copolymers of 2-nitrobenzyl-L-glutamate (ϕNO_2) and glutamic acid has been studied. Previous circular dichroism studies of five copolymers [2] have shown that 2-nitrobenzyl-L-glutamate residues stabilize the α-helical structure. The potentiometric titration curves of these copolymers allowed us to determine the α-helix to random coil transition free energy change of a 2-nitrobenzyl-L-glutamate residue in an aqueous solution of 0.2 M NaCl.

$$\Delta G^0_{hc}\ (\phi NO_2) = +\ 956\ \text{cal/mol}$$

In the present communication, a variation of ΔG^0_{hc} (ϕNO_2) with temperature is reported; so that the related enthalpy change ΔH^0_{hc} (ϕNO_2) and the entropy change, ΔS^0_{hc} (ϕNO_2) can be obtained from the general thermodynamic relation

$$\frac{\Delta G^0_{hc}\ (\phi NO_2)}{T} = \frac{\Delta H^0_{hc}\ (\phi NO_2)}{T} - \Delta S^0_{hc}\ (\phi NO_2)$$

since the variation of ΔG^0_{hc} (ϕNO_2)/T versus 1/T is represented by a linear relationship. Positive values were obtained for ΔH^0_{hc} (ϕNO_2) and ΔS^0_{hc} (ϕNO_2). Therefore, a circular dichroism study was carried out to examine the stability of helical structure with temperature.

2. Materials and methods

Five so-called copolymers O_3, O_6, O_{10}, O_{16} and O_{22} containing 3.5%, 6%, 10%, 16% and 22% of 2-nitrobenzyl-L-glutamate respectively were prepared by a method previously described [2]. These copolymers were studied by circular dichroism [2] at 20, 30, 40, 50, 60, 70 and 80°C with copolymers O_3, O_{10}, O_{16} and O_{22} and by potentiometric titrations at 1, 11, 25, 40 and 55°C with copolymers O_3, O_6, O_{10} and O_{16}. All measurements were made in 0.2 M NaCl solution.

3. Results and discussion

The titration curves have been plotted by using Wada representation [3] in which pK_{eff} = pH –

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*Present address: Institut de Recherches sur le Cancer, B. P. No. 3567, 59020 Lille Cédex France
To whom correspondence should be addressed

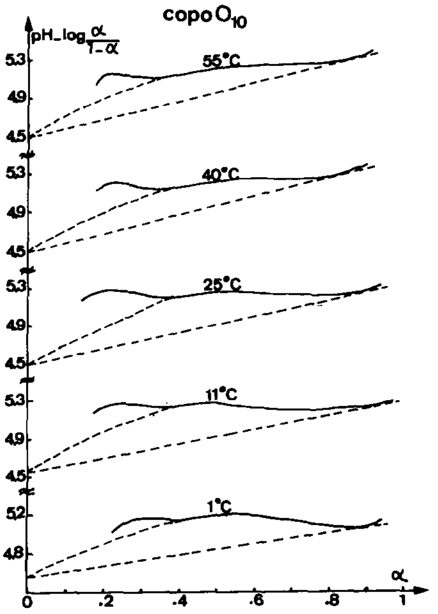

Fig.1. Curves of potentiometric titration in Wada representation, for the copolymer O_{10} and different temperatures in 0.2 M NaCl aqueous solution. The dotted lines represent the extrapolations given the pK_0 value at $\alpha = 0$. (See the text for explanation.)

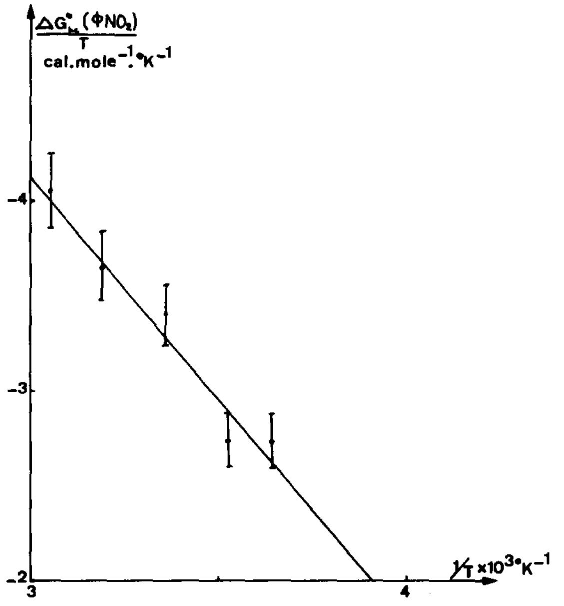

Fig.2. Variation of ΔG^0_{hc} (ϕNO_2)/T versus 1/T ΔG^0_{hc} (ϕNO_2) represents the free energy change of a 2-nitrobenzyl-L-glutamate residue corresponding to the α-helix to random coil transition in 0.2 M NaCl aqueous solution.

$\log(\alpha/1-\alpha)$ is plotted versus α, the degree of ionization of Glu residues. The shape of the curves is approximatively the same for all copolymers as shown in fig.1 for the copolymer O_{10}. Let us explain the significance of these curves: the straight portion at high α values, corresponds to pure random coil conformation. The straight portion at low α values corresponds to pure helical conformation. For very low α values, one observes an aggregation of the copolymer: it corresponds to the little 'tail' which is observed below about $\alpha = 3.5$. The intermediate region – that is between about 4.2 and 7.2 – corresponds to the helix–coil transition. The negative logarithm of the intrinsic dissociation constant, pK_0, is obtained by extrapolation of both linear parts to a common point at $\alpha = 0$. In some cases, the part corresponding to the titration of random coil is not straight but curved and therefore the extrapolation is made from this curve portion as explained in [2]. The helix–coil transition free energy, ΔG^0_{hc} (ϕNO_2), is given by the relationship [4]

$$\Delta G^0_{hc}\,(\phi NO_2) = \frac{RT}{0.434} A$$

in which A is the area bounded by pK_0, the transition region and the extrapolated limbs. ΔG^0_{hc} (ϕNO_2) was determined for each temperature and the variation ΔG^0_{hc} (ϕNO_2)/T versus $1/T$ is given in fig.2. From this linear relationship one obtains ΔH^0_{hc} (ϕNO_2) = 2200 cal/mol and ΔS^0_{hc} (ϕNO_2) = 10.6 cal/mol/deg.

Generally, such large positive values for ΔH^0 and ΔS^0 are not observed for copolymers of L-glutamic acid with a hydrophobic residue. Only Ostroy et al. [5] who have determined ΔH^0 and ΔS^0 by using the Lifson and Roig calculations [6] found two positive values for both ΔH^0 and ΔS^0 corresponding to leucine and valine residues in aqueous solution. Miller and Nylund [7] also obtained slightly positive values for ΔS^0 for the leucine residues from the potentiometric titration curves of random copolymers of L-glutamic acid and L-leucine. On the other hand, Fasman et al. [8,9] observed a stabilization of helical structure with increasing temperature for random copolymers of L-glutamic acid containing about 30% of L-leucine.

Given the positive value of ΔS^0_{hc} (ϕNO_2) we have obtained, the helicity change with the temperature was

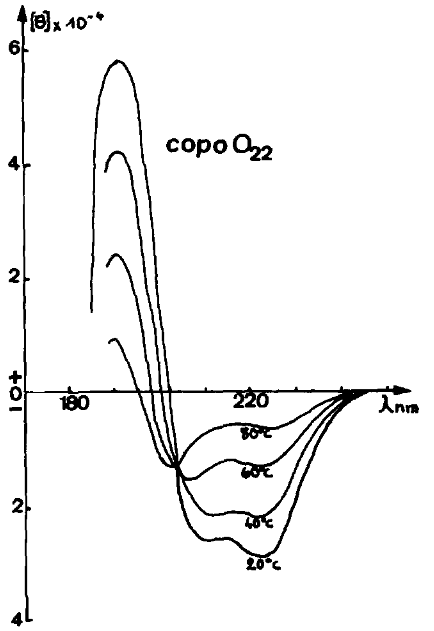

Fig.3. Variation of the dichroic spectra of the copolymer O_{22} at different temperatures in aqueous solution of 0.2 M NaCl.

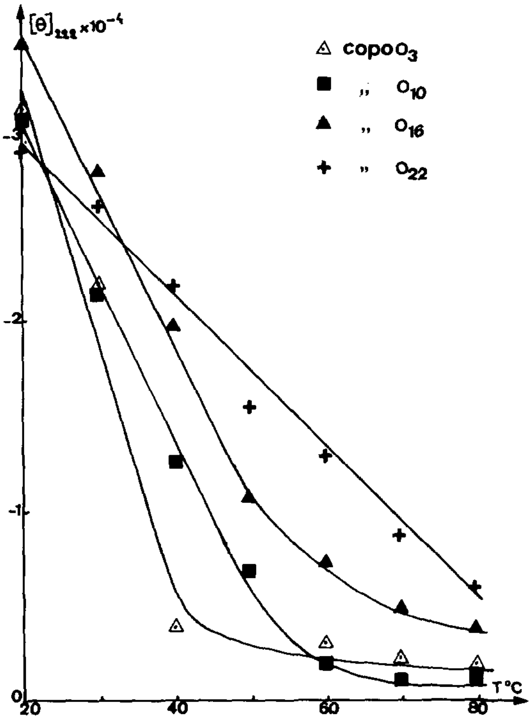

Fig.4. Variation of $[\theta]_{222}$ against temperature for the four copolymers studied.

observed and in fig.3 one can see the variation of the dichroic spectra between 180 and 250 nm. The value of $[\theta]_{222}$ was used to characterize the helicity and fig.4 give the variation of $[\theta]_{222}$ versus temperature for each copolymer. One observes that the stability of the secondary structure increases with the percentage of nitro derivative in copolymer. It is interesting to compare this result with that obtained by Fasman et al. [8,9] for random copolymers of L-glutamic acid and L-leucine (80:20 and 75:25): in a first step – that is between about 20 and 50°C – the authors observe a stabilization of the secondary structure, but when temperature reaches approximatively 50 to 70°C, the stability decreases. This inverse temperature effect has not been observed in the present work.

4. Conclusion

The two main results we have obtained in the study are the following: (1) Nitrobenzyl-L-glutamate stabilizes helical structure in aqueous media; (2) The entropy change, ΔS^0_{hc} (ϕNO_2), is positive.

This is the first time that a positive value of entropy corresponding to the helix–coil transition has been observed for non-ionic residues in a random copolymer containing ionic and non-ionic residues. Generally, previous authors [7–12] have found a value greater than that corresponding to the glutamic acid but near zero, that is slightly negative or slightly positive. Our results concerning the stability of helical structure with increasing temperature are in excellent agreement with the fact that hydrophobic forces play a more important role at elevated temperature.

In a recent work, Ptitsyn et al. [13,14] suggested the presence of a compact structure in a random copolymer of glutamic acid and leucine, evidently due to the hydrophobic interactions of Leu residues. It is possible that such compact structures exist in the copolymers studied here and could be responsible for the large positive values observed for ΔS^0_{hc} (ϕNO_2). Therefore, it can be expected that this important ΔS^0_{hc} (ϕNO_2) should be due to some interactions at long range; in other words the ΔS^0 could reflect a tertiary structure of the copolymer studied. Even if the forces contributing to the stabilization of the copolymer are extremely limited when compared to proteins, one must not lose sight that globular proteins are constituted of about 30% of hydrophobic residues which contribute to the mechanism of protein self-organisation.

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