THERMODYNAMIC PARAMETERS OF HELIX–COIL TRANSITIONS IN POLYPEPTIDE CHAINS

O. B. PTITSYN

Institute of Protein Research, Academy of Sciences of the USSR, Poustchino, Moscow Region, USSR

ABSTRACT

The aim of this paper is to outline new possibilities of more detailed thermodynamic analysis of helix–coil transition parameters, particularly with respect to their splitting into energy and entropy terms. Because of difficulties with the ternary systems, water–organic solvent–polypeptide, or, two organic solvents–polypeptide, caused particularly by the phenomenon of specific sorption of one solvent component, the discussion is restricted to water soluble synthetic polypeptides whose limited range can fairly be extended by copolymerization. It is shown how the combination of data from different experimental sources (optical rotatory dispersion, potentiometric titration curves, intrinsic viscosity, calorimetry) can yield an average number of monomer units in the helical region in the middle of the transition.

The parameters characterizing the initiation and elongation of helical regions determined for a number of polypeptides differing only in their side groups give us valuable information about the intramolecular interactions in polypeptide chains. There is also a significant correspondence between the helix–coil equilibrium constants of natural amino-acids in aqueous media and the distribution of these amino-acids among helical and non-helical regions in globular proteins. This correspondence, together with a treatment of intramolecular interactions, can serve to predict the secondary structure of globular proteins from the primary one.

INTRODUCTION

Experimental and theoretical evaluations of different conformation energies of macromolecules in solution are the basis of the contemporary physics of macromolecules. However the development of the conformational analysis of macromolecules is hindered by a comparative scarcity of experimental data from which it would be possible to draw quantitative information on different conformation energies of macromolecules. The rotation-isomer model of a macromolecule, suggested by Volkenstein[1], made it possible to relate these energies to the average dimensions of macromolecules in solution. The corresponding theory of macromolecules, the working out of which was begun by Birshtein and the author[2] and so splendidly continued by Flory and his collaborators[3], allowed the estimation of the average dimensions of macromolecules, proceeding from the energies of their different conformations, and thus for the first time gave a reliable

basis for an experimental verification of the theoretical conformational analysis of macromolecules. Nevertheless, the number of the experimentally observed quantities is, as a rule, considerably smaller than the number of parameters included in the problem, which demands new approaches to the experimental determination of other quantities depending on the same parameters.

New possibilities are opened in this respect with the study of macromolecules capable of a co-operative rearrangement of their conformation with a change in temperature or in the conditions of the environment. Helix–coil transitions in polypeptide chains are the best-studied type of such rearrangements. A study of polypeptide chains in solution makes it possible to determine, together with the usual parameters such as the average dimensions of macromolecules in the coil-like state, another four important parameters: the differences of energies and entropies connected with initiation and elongation of the helical region of the chain. A determination of these values for a number of synthetic polypeptides, differing only in their side groups, does indeed give unique information on intramolecular interactions in macromolecules. The most important circumstance is that this information directly concerns intramolecular interactions *in polypeptide chains*, which makes it possible to apply the results obtained immediately to the conformational analysis of protein molecules.

Therefore it seems to be quite realistic to switch over from a purely phenomenological description of the helix–coil transition to a detailed analysis of the *nature* of these parameters, the role of energy and entropy terms, the dependence of these parameters on the polypeptide and the solvent, etc. Corresponding experimental investigations are being carried out in a number of laboratories, including our laboratory at the Institute of Protein Research in Poustchino near Moscow. Scheraga and his collaborators in the U.S.A., as well as Birshtein and her co-workers in Leningrad, have undertaken the first steps to work out a molecular theory of the helix–coil transitions in polypeptide chains which directly connects the observed parameters with the energies of different conformations of polypeptide chains. The aim of my report is to outline new possibilities which can be opened with such an approach for a further development of the conformational analysis of polypeptides and other macromolecules.

The phenomenological theory of helix–coil transitions in polypeptide chains, developed by Zimm and Bragg[4] and others (see review in ref. 5), expresses the partition function of a polypeptide chain through the equilibrium constant for the transfer of one monomer unit from the coil-like into the helical region

$$S = \exp(-\Delta F_0/RT) \tag{1}$$

and the cooperativity parameter

$$\sigma = \exp(-\Delta F_{init}(/RT) \tag{2}$$

In equations 1 and 2, ΔF_0 is the change of free energy with an increase in the helical region of the chain by one monomer unit at the expense of the neighbouring coil-like region, and ΔF_{init} is the additional increase of the free energy connected with the initiation of the helical region. From the

viewpoint of the conformational analysis of protein molecules determination of the parameters S and σ in an aqueous medium is, naturally, of the greatest interest. As regards organic solvents, helix–coil transitions have so far been registered only in mixtures of organic solvents either with water or with each other, where the interpretation of the data obtained is complicated by difficulties in the analysis of the three component systems, connected with a specific sorption of one or another component of the solvent and other similar effects. I shall therefore restrict myself to the analysis of the data obtained in an aqueous medium.

The range of water-soluble synthetic polypeptides is rather limited. First of all there are chains containing ionizable groups (poly-L-glutamic acid, poly-L-lysine and others) and performing helix–coil transitions during their ionization. A number of derivatives of poly-L-glutamic acid are also water-soluble, some of which, e.g. poly-N^5-(3-hydroxypropyl)-L-glutamine and poly-N^5-(3-hydroxybutyl)-L-glutamine, perform helix–coil transitions in an aqueous medium with a change in temperature in an accessible temperature range. Finally, it is possible to obtain water-soluble copolymers of the above-mentioned monomers with other monomers (glycine, L-alanine, L-leucine, L-valine, etc.) homopolymers of which do not dissolve in water. A study of such copolymers (block, regular or random) can yield information on the S or σ constants of both their components, including the one which does not form water-soluble polymers itself.

ESTIMATION OF ΔF_0 VALUES

A standard state to which it is convenient to attribute the value of the S parameter is the state of a non-ionized chain. The difference in free energies of the uncharged chain in the helix and in the coil (calculated per monomer) can be estimated from the obvious equation:

$$\Delta F_0 = F^{\text{ioniz}} - F^{\text{ioniz}}_{\text{coil}}, \tag{3}$$

where F^{ioniz} is the free energy of ionization of the real chain, performing a helix–coil transition during ionization, and $F^{\text{ioniz}}_{\text{coil}}$ is the free energy of ionization of the hypothetical chain, retaining the coil-like conformation at all degrees of ionization.

Inasmuch as:

$$F^{\text{ioniz}} = \int_0^1 \frac{\partial F}{\partial \alpha}\,\mathrm{d}\alpha = \int_0^1 \mu(\alpha)\mathrm{d}\alpha = -\,2.3RT\int_0^1 \mathrm{pH}(\alpha)\mathrm{d}\alpha,$$

equation 3 acquires the form[6]:

$$\Delta F_0 = -\,2.3RT\int_0^1 [\mathrm{pH}(\alpha) - \mathrm{pH}_{\text{coil}}(\alpha)]\,\mathrm{d}\alpha, \tag{3'}$$

where $\mathrm{pH}(\alpha)$ is the titration curve of the real chain performing the helix–coil transition, and $\mathrm{pH}_{\text{coil}}(\alpha)$ is the titration curve of the hypothetical chain, retaining the coil-like structure at all degrees of ionization. This curve can

tion curve. In practice it is convenient to use the modified titration curve pK(α), where:

$$pK(\alpha) \equiv pH(\alpha) \pm \log \frac{\alpha}{1-\alpha}$$

(the + refers to polybases, and the − to polyacids) instead of the titration curve pH(α).

As an example the modified titration curves of poly-L-glutamic acid in 0.2 M NaCl at different temperatures, reproduced from our paper[7], are shown in *Figure 1*. It is seen from the *Figure* that the real titration curves in pK(α) coordinates are divided into four regions: the helix titration region

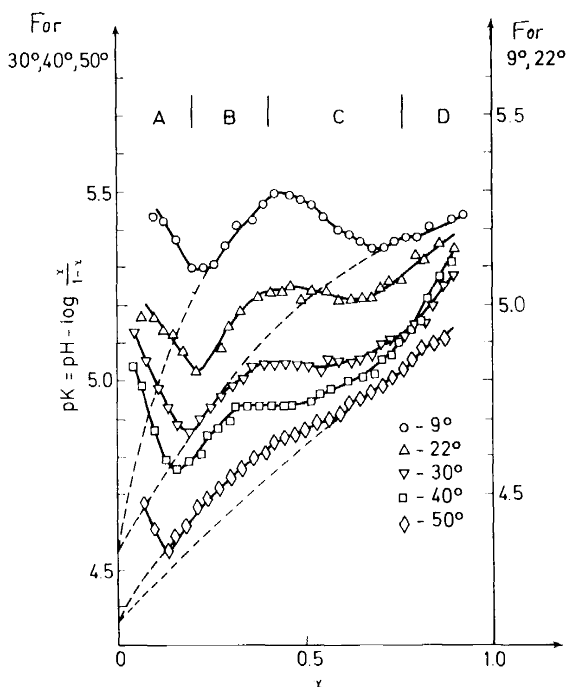

Figure 1. Potentiometric titration curves of poly-L-glutamic acid in 0.2 M NaCl at different temperatures[7]. Meaning of regions A, B, C and D given in text

(B), the coil titration region (D), the helix–coil transition region (C), and, finally, the region of titration of the polypeptide precipitate at a small degree of ionization (A). As a result, not only the coil titration region (D), but also the helix titration region (B) must be extrapolated to $\alpha = 0$ for an evaluation of ΔF_0. The extrapolation cannot be carried out completely unambiguously; however, different, reasonable methods of extrapolation lead to more or less close results. The dependence of ΔF_0 on temperature, plotted according to our data (circles) as well as according to the data of Olander and Holtzer[8] (other symbols) is given in *Figure 2*. The data refer to different ionic strengths, from 0.01 M NaCl (triangles) up to 0.40 M

NaCl (rhombuses), and demonstrate that the dependence of ΔF_0 on the ionic strength is small or absent. The temperature dependence of ΔF_0 is approximated by the straight line $\Delta F_0 = \Delta H_0 - T\Delta S_0$, where $\Delta H_0 = -975$ cal mol^{-1}, and $\Delta S_0 = -2.67$ eu. Very close values ($\Delta H_0 = -1\,000$ cal mol^{-1} and $\Delta S_0 = -2.8$ eu) were also obtained by Miller and Nylund[9] in an analogous manner. A similar value of $\Delta H_0 = -1\,100 \pm 200$ cal mol^{-1} was obtained by Rialdi and Hermans[10] from calorimetric data.

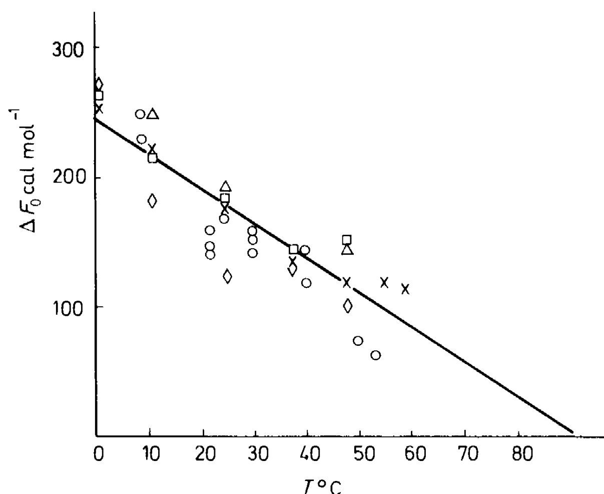

Figure 2. Temperature dependence of ΔF_0 for uncharged poly-L-glutamic acid molecules. ⊙ – 0.20 M NaCl[7]; △ – 0.01. ⊡ – 0.05, × – 0.10. ◇ – 0.40 M NaCl[8]. Straight line correponds to $\Delta F_0 = \Delta H_0 - T\Delta S_0$, where $\Delta H_0 = -975$ cal mole^{-1}, and $\Delta S_0 = -2.67$ e.u.

Figure 3 shows the titration curves obtained by us[11] for poly-L-lysine in 0.2 M NaCl also at different temperatures. The ΔF_0 values obtained are given versus temperature in *Figure 4*; the temperature dependence of ΔF_0 is well approximated by the values $\Delta H_0 = -790$ cal mol^{-1} and $\Delta S_0 = -2.4$ eu, close to the ΔH_0 and ΔS_0 values for poly-L-glutamic acid.

Thus, according to our data and the data of other authors the low stability of the helical state of uncharged molecules of poly-L-glutamic acid and poly-L-lysine in an aqueous medium at room temperature ($\Delta F_0 \simeq -0.1 \sim -0.2$ kcal mol^{-1}) is a result of the compensation of the enthalpy and entropy terms, each of which is higher by an order of magnitude than ΔF_0. However, this is not necessarily so for other polypeptides (see *Table 1*). According to the data of Okita, Teramoto and Fujita[12], the ΔH_0 value for poly-N^5-(3-hydroxypropyl)-L-glutamine is as small as ~ -0.1 kcal mole^{-1} (see also ref. 13). Low ΔH_0 values were also obtained by Scheraga and collaborators for poly-L-alanine (-0.2 kcal mol^{-1})[14] and poly-L-leucine ($+0.1$ kcal mol^{-1})[15] while investigating helix–coil transitions in block copolymers of L-alanine and L-leucine with DL-lysine. The evaluation of ΔH_0 in these cases was made from the dependence of the degree of helicity on temperature and the σ value, determined from the dependence of the degree

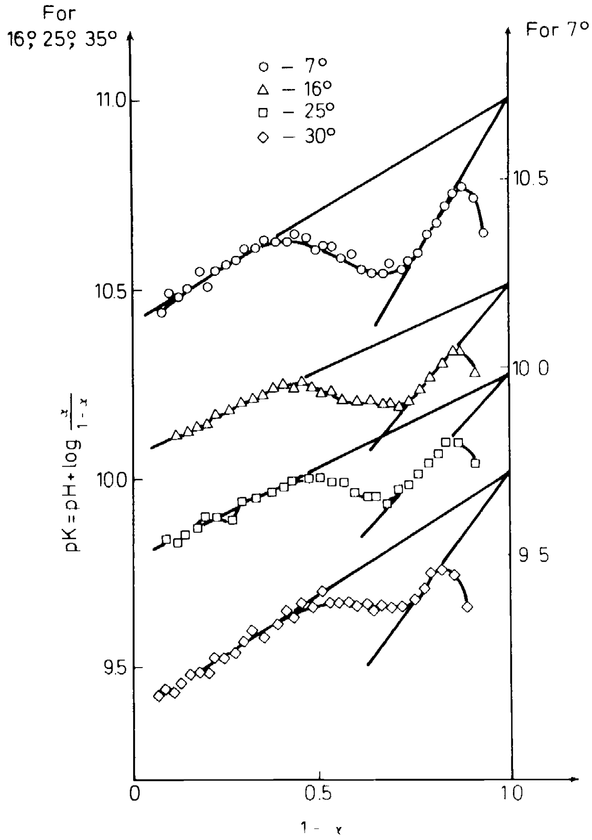

Figure 3. Potentiometric titration curves of poly-L-lysine in 0.2 M NaCl at different temperatures[11]

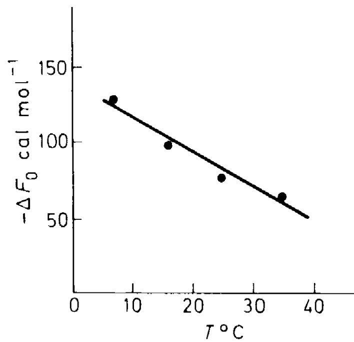

Figure 4. Temperature dependence of ΔF_0 for uncharged poly-L-lysine molecules[11]. Straight line corresponds to $\Delta F_0 = \Delta H_0 - T\Delta S_0$, where $\Delta H_0 = -790$ cal mole^{-1}, and $\Delta S_0 = -2.4$ eu

of helicity on the polymerization degree at a given temperature (see below). Though this estimation is less direct than that derived from the potentiometric titration curves, the conclusion that there is a decrease in the absolute value of ΔH_0 and ΔS_0 and even of a change of sign in ΔH_0 and ΔS_0 in the series of polypeptides considered is, apparently, qualitatively correct. It is

Table 1. Comparison of ΔH_0 and ΔS_0 values for some polypeptides in aqueous media

Polypeptide	Monomer unit	ΔH_0 kcal mol^{-1}	ΔS_0 entropy units
Poly-L-glutamic acid[7,8]	—NH—CH—CO— with side chain CH$_2$—CH$_2$—C(=O)—O—H	−1.0	−2.7
Poly-L-lysine[11]	—NH—CH—CO— with side chain (CH$_2$)$_4$—NH$_2$	—0.8	—2.4
Poly-L-alanine[14]	—NH—CH—CO— with side chain CH$_3$	−0.2	−0.6
Poly-N^5-(3-hydroxy-propyl)-L-glutamine[12]	—NH—CH—CO— with side chain CH$_2$—CH$_2$—CONH—CH$_2$CH$_2$CH$_2$—OH	−0.1	−0.3
Poly-L-leucine[15]	—NH—CH—CO— with side chain CH$_2$—CH(CH$_3$)$_2$	+0.1	+0.7 ~ ~ +1.0

interesting to note that this series of polypeptides approximately coincides with the series put in order of increase of the hydrophobic nature of their side groups. This may mean that hydrophobic interactions of side-groups with the main chain or with each other are stronger in the helical state of the chain than in the coil-like, which leads to an increase in both the energy and the entropy of the helix in comparison with the coil.

ESTIMATION OF ΔF_{init} VALUE

Further important information on intramolecular interactions in polypeptide chains can be received from the cooperativity parameter σ and its dependence on temperature. A method for estimating the σ parameter for polypeptide chains, performing helix–coil transitions during ionization, was suggested by Nagasawa and Holtzer[16]. This method is based on the obvious equation:

$$\Delta F(\mathrm{pH}) = \Delta F(\mathrm{pH}_t) + \int_{\mathrm{pH}_t}^{\mathrm{pH}} \frac{\partial \Delta F}{\partial(\mathrm{pH})} \mathrm{d(pH)}. \tag{4}$$

Taking into account that for helix–coil transitions[17]

$$\ln s \equiv -\frac{\Delta F}{RT} = \sigma^{\frac{1}{2}}(2\vartheta - 1)(1 - \vartheta)^{-\frac{1}{2}}\vartheta^{-\frac{1}{2}} \tag{5}$$

and that in the middle of the transition (at pH_t) $\Delta F = 0$, we obtain (see ref. 17)

$$(2\vartheta - 1)(1 - \vartheta)^{-\frac{1}{2}}\vartheta^{-\frac{1}{2}} = -\sigma^{-\frac{1}{2}} \int_{\mathrm{pH}_t}^{\mathrm{pH}} \frac{\partial \Delta F}{\partial(\mathrm{pH})} \mathrm{d(pH)}$$

$$= \pm 2.3\, \sigma^{-\frac{1}{2}} \int_{\mathrm{pH}_t}^{\mathrm{pH}} \Delta\alpha(\mathrm{pH})\mathrm{d(pH)}$$

$$\simeq \pm 2.3\, \nu \Delta\alpha(\mathrm{pH}_t)(\mathrm{pH} - \mathrm{pH}_t), \tag{6}$$

where $\Delta\alpha(\mathrm{pH})$ is the difference in degree of ionization of the coil and the helix at a given pH, and the signs + and − refer to the polybases and polyacids correspondingly. The value $\sigma^{-\frac{1}{2}}$ in the right-hand part of equation 6 is equal to the average number of monomer units in the helical and coil-like region of the chain in the middle of the transition:

$$\nu = \sigma^{-\frac{1}{2}}. \tag{7}$$

It follows from equation 6 that the ν value can be obtained from the combination of the data on dispersion of optical activity (which give the helicity degree ϑ) and potentiometric titration (giving $\Delta\alpha(\mathrm{pH})$). The physical sense of this method is reduced to the fact that the sharpness of the dependence $\vartheta(\mathrm{pH})$ is determined by the difference of the number of protons bound in the middle of the transition to *the cooperative region of the chain* (consisting of ν monomers) in the coil-like and helical states. Therefore, knowing the sharpness of the transition from the data on optical activity and the difference of the number of protons bound to one monomer from the potentiometric titration data it is possible to determine the number of monomers in the cooperative region, i.e. $\nu = \sigma^{-\frac{1}{2}}$.

The second method of determining the parameter $\nu = \sigma^{-\frac{1}{2}}$ is based on the theory of dimensions of polypeptide chains in the region of the helix–coil transition, developed by the author and A. M. Skvortsov[18–20]. Accord-

ing to this theory (including Hagnauer and Miller's correction[21] which takes into account the influence of long-range interactions):

$$f(\vartheta) \equiv \frac{\langle h^2(\vartheta)\rangle}{\langle h^2_{\text{coil}}\rangle} = 1 - \vartheta + 2\nu \frac{l^2_{\text{helix}}}{\alpha^2_{\text{coil}}\, l^2_{\text{coil}}} \vartheta \Big/ \sqrt{\left(\frac{\vartheta}{1-\vartheta}\right)}, \tag{8}$$

i.e. the mean square of the distance between the ends of the chain $\langle h^2(\vartheta)\rangle$ increases at a given ϑ with an increase of the transition cooperativity (i.e., with an increase of the average number of monomers in the helical region). In equation 8 (valid at ϑ not very close to unity and $1 \ll \nu \ll n$), $l_{\text{helix}} = h_{\text{helix}}/n = 1.5$ Å, $l_{\text{coil}} = (\langle h^2_{\text{coil}}\rangle_0/n)^{\frac{1}{2}} = 11.4 \pm 0.4$ Å[22], and $\alpha^2_{\text{coil}} = \langle h^2_{\text{coil}}\rangle / \langle h^2_{\text{coil}}\rangle_0$ (the zero index indicates the absence of long-range interactions). Relative dimensions of the chains at different ϑ can (at ϑ not very close to one) be obtained by the well-known Flory equation from the relative values of intrinsic viscosities:

$$f(\vartheta) \equiv \frac{\langle h^2(\vartheta)\rangle}{\langle h^2_{\text{coil}}\rangle} \simeq \left(\frac{[\eta](\vartheta)}{[\eta]_{\text{coil}}}\right)^{\frac{2}{3}}. \tag{9}$$

We have determined[7, 11] the ν values for poly-L-glutamic acid and poly-L-lysine at different temperatures in an aqueous medium by both the methods mentioned. As an example, the dependences of $[\alpha]_{546}$ and $[\eta]$ versus pH for poly-L-glutamic acid in 0.2 M NaCl at different temperatures are given in *Figures* 5 and 6. The dependence of $[\eta](\vartheta)/[\eta]_{\text{coil}}$ versus the helicity degree, calculated in the usual manner from the data on optical activity[7], is given in *Figure 7*. The coincidence of the curves $[\eta](\vartheta)/[\eta]_{\text{coil}}$ at all temperatures shows by itself that the parameter ν (i.e. σ) practically does not

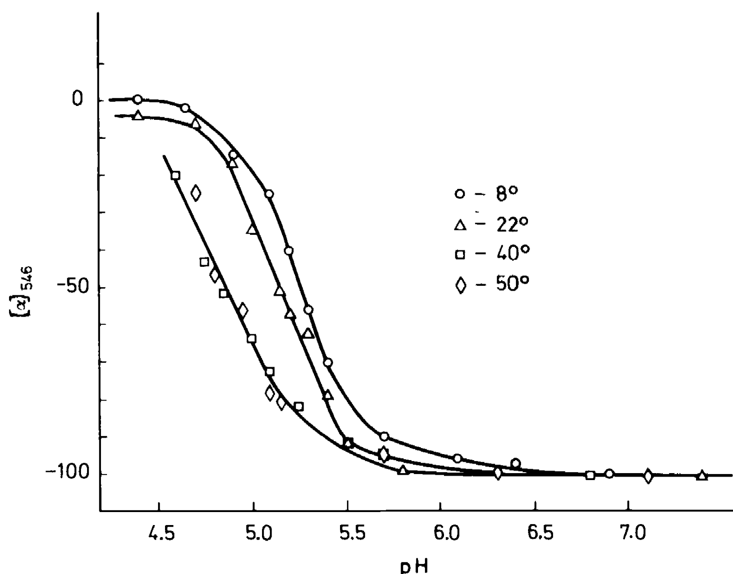

Figure 5. Dependence of specific rotation $[\alpha]_{546}$ on pH for poly-L-glutamic acid in 0.2 M NaCl at different temperatures[7]

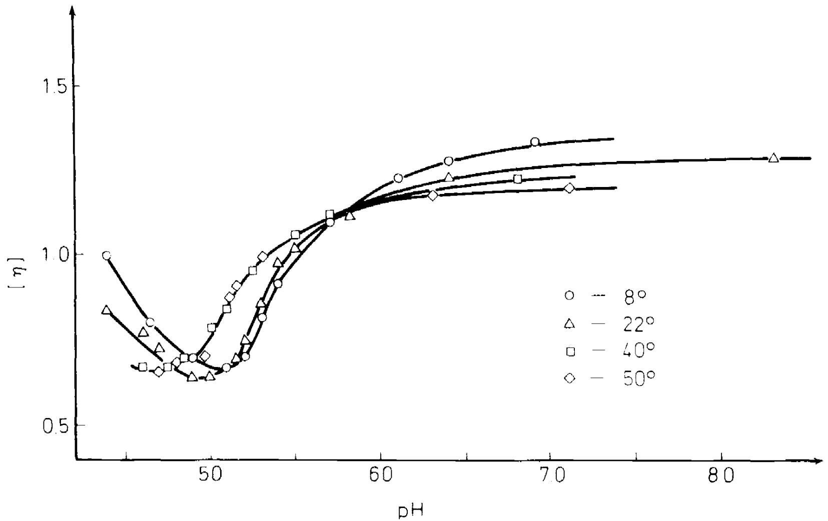

Figure 6. Dependence of characteristic viscosity $[\eta]$ on pH for poly-L-glutamic acid in 0.2 M NaCl at different temperatures[7]

depend on temperature. This conclusion is corroborated by quantitative determination of ν from the data on the sharpness of the transition (from equation 6) as well as the chain dimensions in the transition region (from equation 8). Analogous results for poly-L-lysine[11] are given in *Figures 8*, *9* and *10* correspondingly. *Table 2* lists all the ν values obtained by us for these two polypeptides at different temperatures and ionic strengths in an aqueous medium. It is seen from the table that for both polypeptides the value ν is practically independent of temperature and ionic strength, and is equal to 20 ± 2 for poly-L-glutamic acid and 21 ± 2 for poly-L-lysine. The

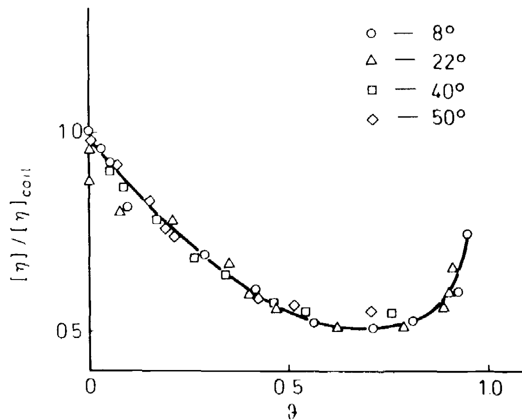

Figure 7. Dependence of $[\eta]/[\eta]_{coil}$ on the helicity degree ϑ for poly-L-glutamic acid in 0.2 M NaCl at different temperatures[7]

Table 2. Values of cooperativity parameter $v = 1/\sqrt{\sigma}$ for poly-L-glutamic acid and poly-L-lysine in aqueous media

Poly-L-glutamic acid

Solvent	Temperature °C	$v = \sigma^{-\frac{1}{2}}$ from the sharpness of transition	$v = \sigma^{-\frac{1}{2}}$ from the sizes of the chains
0.02 M NaCl	22	—	25
0.2 M NaCl	8	—	19
	12	22	—
	22	22	19
	40	18	19
	50	23	19
1 M NaCl	22	—	21
2 M NaCl	22	—	22

mean value of $v = 20 \pm 2$

Poly-L-lysine

Solvent	Temperature °C	from the sharpness of transition	from the sizes of the chains
0.2 M NaCl	8	22	—
	16	22	21
	25	21	20
	35	22	—
0.2 M NaBr	22	22	21
1 M KCl	22	—	22

mean value of $v = 21 \pm 2$

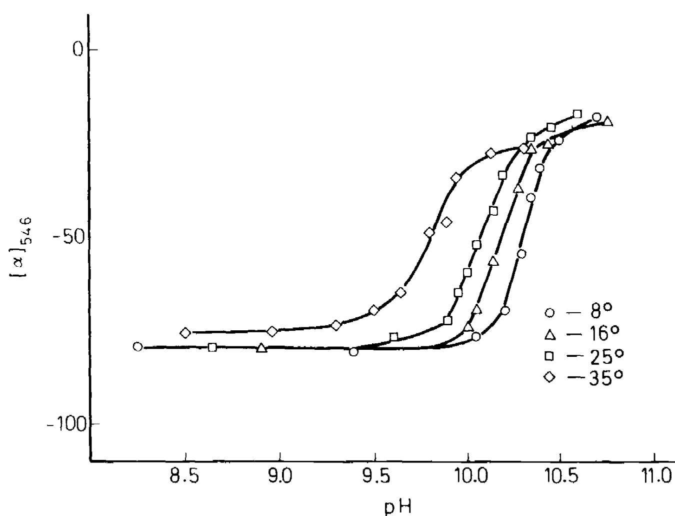

Figure 8. Dependence of specific rotation $[\alpha]_{546}$ on pH for poly-L-lysine in 0.2 M NaCl at different temperatures[11]

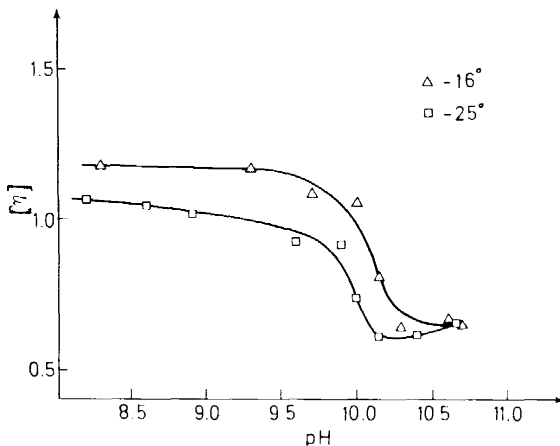

Figure 9. Dependence of characteristic viscosity $[\eta]$ on pH for poly-L-lysine in 0.2 M NaCl at different temperatures[11]

corresponding σ values are equal to $(2.5 \pm 0.5)\cdot 10^{-3}$ and $(2.3 \pm 0.5)\cdot 10^{-3}$ at all the temperatures and ionic strengths. This means that the free energy of initiation $\Delta F_{init}/RT = 6.0 \pm 0.2$ in the investigated temperature range from 8° to 50°C for both polypeptides. In other words in both polypeptides $\Delta S_{init} \simeq -12$ eu, and ΔH_{init} is very small (its absolute value in any case does not exceed a few hundreds of cal mol^{-1}).

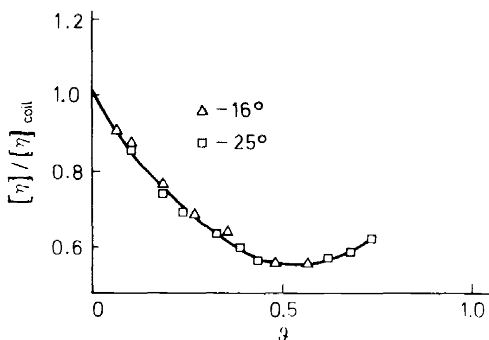

Figure 10. Dependence of $[\eta]/[\eta]_{coil}$ on the helicity degree ϑ for poly-L-lysine in 0.2 M NaCl at different temperatures[11]

It is interesting to compare these data with the results obtained by other authors for other water-soluble polypeptides. All these data are obtained from the dependence of the degree of helicity ϑ on the polymerization degree n utilizing the helix–coil transition theory for finite chains. Okita, Teramoto and Fujita[12] have recently obtained a simple and convenient equation for determining v from the $\vartheta(n)$ dependence:

$$\vartheta_n = \vartheta - 2\vartheta\{\vartheta(1-\vartheta)\}^{\frac{1}{2}}\frac{v}{n-2} \tag{10}$$

where ϑ_n is the degree of helicity of the chain consisting of n monomers, and ϑ is the degree of helicity of the infinite chain (practically $\vartheta = \vartheta_n$ at $n \gg \nu$). In this manner Okita, Teramoto and Fujita determined ν-values for poly-N^5-(3-hydroxypropyl)-L-glutamine in water at different temperatures from 5° to 40°C. They established that $\nu = 56 \pm 4$ also without a clearly expressed temperature dependence. An analogous value of $\nu = 59$ was obtained for this polypeptide also in ref. 13 at room temperature.

Scheraga and his collaborators made an effort to estimate the ν parameter for poly-L-alanine[14] and poly-L-leucine[15] from the temperature of helix–coil transitions in block copolymers containing central blocks from L-alanine and L-leucine and flanking blocks from DL-lysine. The dependence of the degree of helicity on the dimensions of the central block was also used, but the ν parameter was regarded *a priori* to be independent of temperature. This method is open to criticism, since a decrease in the helicity degree of the central block with a decrease of its size can be stipulated not only by comparatively large ν values but also by an increase of the role of repulsion of charged flanking blocks from DL-lysine. The values of ν obtained are approximately equal to 80 for poly-L-alanine[14] whereas the experimental data on poly-L-leucine[15] satisfy any ν values lying between 20 and 90. Thus, at present it is still early to make a general conclusion concerning the possible dependence of ν on the polypeptide nature in an aqueous medium, though an impression is created that poly-L-glutamic acid and poly-L-lysine have a somewhat lesser degree of cooperativity than other investigated polypeptides.

THE NATURE OF COOPERATIVITY OF HELIX–COIL TRANSITION IN POLYPEPTIDE CHAINS AND INTRAMOLECULAR INTERACTIONS

It is possible, *a priori*, to indicate two reasons why cooperativity of helix–coil transitions in polypeptide chains should occur:

1. The formation of the first hydrogen bond in the α-helix involves fixing the conformations of three monomer units, and the formation of every following hydrogen bond requires the fixation of only one monomer unit. Thus, the initiation of the helical region requires a fixing of conformations of two 'excess' monomer units.

2. Intramolecular interactions in a polypeptide chain can be different in the middle of the helix and at its ends, with the possibility that the energy of these interactions at the ends of the helix is greater than in the middle.

The first of these two mechanisms was postulated by Zimm and Bragg[4] who proposed the first phenomenological theory of helix–coil transitions, and for a long period this mechanism was considered to be the only or, in any case, the basic one. However, the latest attempts at building up a molecular theory of helix–coil transitions[23–26] have advanced this problem in a new way. According to refs. 23 and 24 an essential part of the increase in the free energy with initiation of the helical region in uncharged polypeptide chains must be connected with the dipole–dipole interaction of non-neighbouring peptide groups. These interactions stabilize the helix in a sufficiently long helical region[27]. However at the ends of the helical region these inter-

actions cannot be completely realized, due to which a peculiar 'surface tension' appears at the boundaries of the helical region (the energy of a monomer unit at the ends of the helix is greater than in its middle).

It is evident that the first of the above-mentioned mechanisms of co-operativity is mainly of an entropy origin, and the second mainly of an energy origin. Therefore the experimental proof of the entropy nature of co-operativity in poly-L-glutamic acid and poly-L-lysine contradicts the conclusions of the theory on the considerable role of interactions of non-neighbouring peptide groups. Actually, the $\Delta H_{init} \simeq 4$–6 kcal mol^{-1} values for polyglycine and poly-L-alananine were obtained in refs. 23 and 24. Inasmuch as these values are mainly due to the interaction of peptide groups of the main chain and not of the side groups, this contribution to ΔH_{init} must be preserved also for other polypeptides. At the same time such ΔH_{init} values correspond to an approximately two-fold decrease of v in a temperature range of 8–50°C, which completely contradicts our experimental data as well as the data of Okita, Teramoto and Fujita[12].

Thus, it is to be concluded that the usual method of calculating electrostatic interactions in polymer chains (Coulomb interaction of partial charges with the value of the dielectrical constant $\varepsilon = 4$) applied also in papers[23, 24] leads to a considerable overestimation of the role of electrostatic interactions of far groups in aqueous media. Actually, it is well known that the interaction of two charges, situated near the interface of media with different dielectric constants, cannot be described by a simple Coulomb's law: the effective value of the dielectric constant increases with an increase of the distance between charges. The latest versions of the molecular theory of helix–coil transitions in polypeptide chains[25, 26] take this circumstance into account, merely rejecting the electrostatic interaction of peptide groups sufficiently far along the chain. In the paper of Birshtein and her co-workers[25] the electrostatic interaction of neighbouring peptide groups only was taken into consideration, as a result of which $\Delta H_{init} < 1$ kcal mol^{-1} was obtained (for poly-L-alanine). In a new version of Scheraga's paper[26] the electrostatic interaction of all spatially close peptide groups (with a distance not exceeding ~ 6 Å) is taken into account, which in the helical state includes four neighbours of a peptide group on each side. The value $\Delta H_{init} \simeq 2$ kcal mol^{-1} is obtained for poly-L-alanine.

The case is much better with an interpretation of the experimental data on the entropy of initiation of the helical regions. In communications 24–26 values of the entropy of initiation from -10 up to -12 eu were obtained for poly-L-alanine, which is very close to our experimental data for poly-L-glutamic acid and poly-L-lysine (-12 eu). A somewhat greater value (~ -16 eu) follows from the data of Okita, Teramoto and Fujita[12] for poly-N^5-(3-hydroxypropyl)-L-glutamine, if it is assumed that in their case the cooperativity is also completely of an entropy character. It was shown in papers 24–26 that the limitation of conformations of monomer units at the ends and in the middle of helical regions approximately coincides, so that:

$$\Delta S_{init} \simeq 2\Delta S_{conf}, \tag{11}$$

where ΔS_{conf} is the change of entropy of a monomer unit at its incorporation into the helical region. Thus, for poly-L-glutamic acid and poly-L-lysine in water–salt solutions $\Delta S_{conf} = -6$ eu, which corresponds to an approximately twenty-fold decrease of the range of angles in which the internal rotation is realized. The closeness of this result to the predictions of the theory for poly-L-alanine means, on the one hand, that the main role in the entropy effect is played by the limitation of the conformation of the main chain and not of the side groups, and on the other, that the existing methods of theoretical conformational analysis satisfactorily describe the conformational freedom of monomer units of a polypeptide chain in the coil-like and helical states.

The free energy of helix stabilization can be presented as:

$$\Delta F_0 = \Delta F_{inter} - T\Delta S_{conf}, \qquad (12)$$

where ΔF_{inter} is the difference of free energies of the polypeptide–solvent system for the helical and coil-like states of the polypeptide chain due to the formation of intramolecular hydrogen bonds in the helix and to a change of other intra- and intermolecular interactions. The substitution of $\Delta S_{conf} \simeq -6$ eu and $\Delta F_0 \simeq -0.1 \sim -0.2$ kcal mol^{-1} into equation 12 gives $\Delta F_{inter} \simeq -2$ kcal mol^{-1}. Thus, the low stability of the helical state of the polypeptide chain in an aqueous medium is a result of an almost complete compensation of two major values: the gain of the free energy of the helix ΔF_{inter} (caused by the formation of intramolecular hydrogen bonds and the change of other interactions) and the loss of the free energy of the helix $T\Delta S_{conf}$ (connected with the fixation of conformations of monomer units).

In conclusion of this section some words can be said on possible reasons for differences in the cooperativity parameter values in poly-L-glutamic acid and poly-L-lysine on the one hand and poly-N^5-(3-hydroxypropyl)-L-glutamine and, probably, poly-L-alanine on the other. The fact is that in the first group of polypeptides the experimentally determined σ values refer to the partially charged state of the chain, corresponding to the ionization of approximately half of all the ionizable groups. In partially ionized chains the electrostatic repulsion of groups close along the chain is stronger in the helix than in the coil (which, as is known, explains the helix–coil transition during ionization). This repulsion is, naturally, less at the ends of the helical region than in its middle, which decreases the free energy of monomer units at the ends of the helix. This effect must lead to a decrease of mean sizes of helical regions, i.e. to a decrease in cooperativity.

THERMODYNAMIC PARAMETERS OF HELIX–COIL TRANSITIONS AND THE SECONDARY STRUCTURE OF GLOBULAR PROTEINS

In conclusion, let us consider the relation of thermodynamic parameters of helix–coil transitions in synthetic polypeptides to the secondary structure of globular proteins. A globular protein is a three-dimensional cooperative system, the state of every region of which is, generally speaking, determined both by local interactions in this region and by long-range interactions of monomer units of this region with monomer units, close in space but remote

along the chain. At the same time, thermodynamic parameters of helix–coil transitions in polypeptide chains, having no tertiary structure, are practically completely determined by local interactions. Therefore, it is not clear, *a priori*, to what extent the helicity of one or another region of the globular protein chain is connected with the helix–coil transition equilibrium constants for amino-acids contained in this region.

In order to clarify this question let us compare the equilibrium constants s for natural amino-acids, determined from synthetic polypeptides, with the distribution of these amino-acids among helical and non-helical regions in globular proteins with a known spatial structure.

Table 3. Comparison of distribution of amino-acids among helical and non-helical regions in 9 globular proteins with the equilibrium constants of these amino-acids for helix–coil transitions

Amino -acid	N	n_{helix}	$\vartheta = \frac{n_{\text{helix}}}{N}$	$S = \exp\left(-\frac{\Delta F_0}{RT}\right)$	$\vartheta_0 = \frac{S}{1+S}$
Glu	62	39 ± 4	0.63 ± 0.06	1.28	0.56
Ala	165	94 ± 6	0.57 ± 0.04	1.10	0.52
His	54	30 ± 4	0.56 ± 0.07	>1	>0.50
Leu	136	70 ± 6	0.52 ± 0.04	1.30	0.56
Met	20	10 ± 2	0.50 ± 0.10	>1	>0.50
Trp	34	17 ± 3	0.50 ± 0.10		
Gln	65	30 ± 4	0.46 ± 0.06	<1.28	<0.56
Val	136	60 ± 6	0.44 ± 0.04	(<1)	(<0.50)
Lys	106	47 ± 5	0.44 ± 0.05	1.14	0.53
Phe	56	21 ± 4	0.38 ± 0.07	>1.28 (<1)	>0.56 (<0.50)
Ile	70	23 ± 4	0.33 ± 0.06		
Asp	76	25 ± 4	0.33 ± 0.05	<1	<0.50
Thr	103	34 ± 5	0.33 ± 0.05		
Arg	53	15 ± 4	0.28 ± 0.08		
Ser	158	45 ± 6	0.28 ± 0.04	(<1)	(<0.50)
Pro	64	16 ± 4	0.25 ± 0.06	≪1	≪0.50
Asn	93	23 ± 5	0.25 ± 0.05		
Gly	157	38 ± 6	0.24 ± 0.04	0.59	0.38
Cys	37	8 ± 3	0.22 ± 0.08	(<1)	(<0.50)
Tyr	69	13 ± 4	0.19 ± 0.06	(<1)	(<0.50)
Total number	1714	658 ± 20	0.38 ± 0.01		

Table 3 lists the general number N of residues of every amino-acid in 9 globular proteins (myoglobin, α- and β-chains of haemoglobin, lysozyme, ribonuclease, α-chymotrypsin, papain, subtilysine and carboxypeptidase), as well as the number n_{helix} of these residues, included according to x-ray diffraction data, in helical regions (the error in determination of n_{helix}, mentioned in the table, represents the dispersion of normal distribution for a given number of amino-acids). According to these data, the share of every amino-acid in helical regions $\vartheta = n_{\text{helix}}/N$ varies from almost $\frac{2}{3}$ for glutamic acid up to $\sim\frac{1}{5}$ for tyrosine. Column 5 of the table lists the values of the equilibrium constant S between the helix and the coil for a given amino-acid, determined from the data on synthetic polypeptides in an aqueous medium, and column 6 gives the $\vartheta_0 = S/(1 + S)$ values equal to

shares of a given amino-acid in the helical regions under conditions of non-cooperative distribution of every amino-acid among helical and non-helical regions in correspondence with the equilibrium constant S (for the sake of simplicity we neglect here the presence of regions with the β-structure). The S values, following from the ΔH_0 and ΔS_0 values, listed in *Table 1*, are taken for Glu, Ala, Leu and Lys; for Gly the value $S = 0.59$ (corresponding to $\vartheta_0 = 0.38$) has been recently obtained by Scheraga and his collaborators[27] from data on copolymers of Gly with hydroxybutylglutamine. For other amino-acids the S estimates were used from the top or bottom based on the following facts: poly-L-histidine can[28] and poly-L-aspartic acid cannot[29] be spiralized in an aqueous medium; the incorporation of methionine into copolymers increases the degree of their helicity[30]; copolymers of Gln with Glu have a smaller degree of helicity than polyglutamic acid[31]; poly-L-proline cannot form α-helices. If the amino-acid forms a polypeptide having a β-structure in aqueous medium (Val[32], Ser[33], Cys[34]†, Tyr[35]), then the values of $S < 1$ and $\vartheta_0 < 0.50$ are conditionally indicated for it (these values are shown in brackets, since it only follows from the experimental data that $S_\alpha < S_\beta$, which does not necessarily mean that $S_\alpha < 0.50$). Different authors[36, 37] have obtained different results for Phe and the *Table* lists both results.

It can be seen that a remarkable correspondence exists between helix–coil equilibrium constants for natural amino-acids in an aqueous medium and the distribution of these amino-acids among helical and non-helical regions in globular proteins (cf. columns 4 and 6 of the *Table*). Whatever the reason for this correspondence, it promises a hope of successful attempts in predicting the secondary structure of globular proteins from their primary structure by taking into account only local interactions. Indeed, an attempt of such a prediction of regions with the helical, as well as with the β-structure in globular proteins, undertaken recently by the author together with Finkelstein[38], led to successful results in almost 80% of the cases.

It would be dangerous to conclude from this that local interactions play the main role in coding the secondary structure of globular proteins. It is well known that the disruption of the tertiary structure or fragmentation of proteins is accompanied by a sharp decrease of their secondary structure which is thus to a very considerable extent determined by long-range interactions as well. An alternative method of predicting the helical structure, recently developed in our laboratory by Lim[39] and based on taking into consideration only long-range interactions, gives even better results than the method based on taking into account only local interactions. From our point of view, the presence of a far-reaching correlation between the helicity of one or another region of a protein molecule and the helix–coil equilibrium constants for amino-acids contained in this region is explained by a remarkable coordination between long-range and local interactions in globular proteins. In other words, if any region of a protein molecule should be helical from the viewpoint of the globule architecture as a whole, it must simultaneously have an increased content of amino-acids with sufficiently great equilibrium constants S between the helix and coil. It

† The data of paper[34] refer to the derivative of Cys.

might be assumed that the presence of such a remarkable coordination between long-range and local interactions in globular proteins is a result of biological evolution, in the course of which such amino-acids sequences were selected for which the indicated coordination occurs.

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