

Stability of α -Helical Structure of Poly(L-alanine)

Kazuhiko KOSUGE, Masako FUJIWARA, Yoshinori ISOGAI,
and Nobuhiko SAITÔ

Department of Applied Physics, Waseda University, Tokyo, Japan.

(Received April 21, 1972)

ABSTRACT: The intramolecular interaction energy and the structure of interactions in poly(L-alanine) were calculated in the region near the α_R -helix with the semiempirical potential functions of nonbonded, electrostatic, rotational, and hydrogen-bonding interactions. In the conformation of the α_R -helix of poly(L-alanine) the nonbonded interaction makes the most dominant contribution to the total energy of the conformation and the hydrogen-bonding energy is almost its 1/7. However there exists between a peptide pair separated by three peptide units a large interaction energy which comes predominantly from the hydrogen-bonding interaction. On the contrary the nonbonded interaction exists mostly between nearest neighbor peptide units. On deforming the conformation slightly from the α_R -helix, this hydrogen-bonding interaction disappears, although the other kinds of interaction remain almost unchanged. Spatially specific hydrogen-bonding fastens the polypeptide chain as a clamp only when the peptide chain takes certain special conformations. Thus it can be concluded that the hydrogen bond is very important for the stability of the α -helix, and it plays an especially essential role for the cooperative helix—coil transition. It is also emphasized that the structure of interaction energy is important for the study of the protein conformation.

KEY WORDS Poly(L-alanine) / α -Helix / Stability / Helix—Coil
Transition / Cooperativity / Structure of Interaction Energy /
Hydrogen-Bonding /

In the cooperative helix—coil transition of polypeptides the hydrogen bond is assumed to play an important role, as one can see in the various theories of helix—coil transition such as developed by Miyake,¹ Zimm and Bragg² or Lifson and Roig.³ On the other hand according to the calculation of interaction energy in polypeptides by Ooi, *et al.*,⁴ the most dominant contribution comes from nonbonded interaction and the hydrogen-bonding energy is about its 1/4 at the α -helix conformation of polyalanine as given in Table I. Further, they showed that the interaction energy is minimum at the right-handed α -helix (α_R) conformation, and moreover that it is still minimum without the hydrogen bond at almost the same conformation. Now can we say that polyalanine is stable and undergoes a similar helix—coil transition even if the hydrogen bonds are missing? Further, have we to modify the conventional theory of helix—coil transition so as to take account of the nonbonded energy as well? To answer these

questions, the mere presence of the energy minimum is not sufficient. In the present paper we shall discuss the stability of poly(L-alanine), as a simplest example, with the intention to apply our line of thought to real protein molecules. Improvements of energy calculation, especially that of electrostatic and hydrogen-bonding energies are attempted, and we obtain results slightly different from but essentially similar to that of Ooi, *et al.*⁴ In addition, we

Table I. Conformational energy of an α_R -helix of poly(L-alanine)

	Energy, kcal/mol-res	
	Ooi, <i>et al.</i>	Ours
Rotational	0.49	0.58
Nonbonded	-5.99	-7.15
Electrostatic	-1.10	2.60
Hydrogen bond	-1.74	-1.02
Total	-8.34	-5.00

find that the nonbonded energy exists almost exclusively between nearest neighbor peptide units, but it is cancelled to a large extent by the positive electrostatic energy, and that the hydrogen bonding fastens the polypeptide chain. Our conclusion is that the hydrogen bond plays an important part in the stability, even though its contribution to the total energy is smaller than that of nonbonded energy.

METHOD OF CALCULATION

Geometrical Description of Polypeptides

The conformation of a molecule with N atoms has $(3N-3)$ degrees of freedom. We consider here a poly(L-alanine) molecule composed of ten peptides units, and each peptide unit has twelve atoms. To decrease the degrees of freedom and to simplify the description of the three dimensional structure of poly(amino acids) it is assumed that bond lengths and bond angles are kept constant and the peptide bonds are in the *trans* planar conformation. For convenience we take a peptide unit instead of amino residue as an element of the poly(L-alanine) chain; thus the chemical composition of the poly(L-alanine) is slightly different from the real molecule. The C terminal of the molecule we considered is not the carboxy group

$$\begin{array}{c} \text{O} \\ || \\ -\text{C}-\text{NH}_2 \end{array}$$

but $-\text{C}-\text{NH}_2$, and the N terminal is not NH_2 but C_1^α atom. The difference of interaction energy coming from this simplification is not large. Under these conditions it is possible to describe the three-dimensional structure of the poly(amino acid) chain in terms of a set of dihedral angles φ_i and ψ_i along the $\text{N}-\text{C}_i^\alpha$ bond and the $\text{C}_i^\alpha-\text{C}'$ bond of i -th C^α carbon re-

spectively, and internal rotation angles χ_{ij} of its side chain in accordance with the nomenclature proposed by Edsall, *et al.*⁵ Table II shows the fixed values of bond lengths and bond angles, and these are the same as the ones adopted by Ooi, *et al.*,⁴ except the bond angle $\tau(\text{N}-\text{C}^\alpha-\text{C}')$ which is taken as 112.5° , in place of 109.5° , since the average value of this angle in real protein was found to be 112.5° from the data of X-ray analysis of myoglobin and lysozyme.⁶

The position of all the atoms of a poly(L-alanine) can be determined in terms of the given set of $(\varphi_i, \psi_i, \text{ and } \chi_{ij})$, by the method of iteration of coordinate transformations from the C terminal peptide unit to N terminal peptide unit, which was developed by Ooi, *et al.*,⁴ and used for protein conformation by Nishikawa, *et al.*⁷

Energy Functions

The electrostatic, nonbonded, rotational and hydrogen-bonding interactions are taken into account to calculate the intramolecular energy. The hydrophobic interaction is not considered here.

The nonbonded interactions are calculated between all the pairs of atoms by means of modified Lennard-Jones 6-12 potential functions which are cut off for the distance greater than 7 \AA and are used in ref 4. The energy between these pairs whose distances are kept constant with the variation of φ_i, ψ_i and χ_{ij} is not calculated.

The rotational energies around the $\text{C}^\alpha-\text{C}'$ and $\text{N}-\text{C}^\alpha$ bonds of the backbone chain and the $\text{C}^\alpha-\text{C}^\beta$ bonds of the side chains are calculated by the same three-fold potential functions as those of Ooi, *et al.*⁴

Table II. Bond lengths and bond angles

Bond	Lengths, \AA	Bond angle	Value	Bond angle	Value
$\text{C}^\alpha-\text{C}'$	1.53	$\tau(\text{N}, \text{C}^\alpha, \text{C}')$	112.5°	$\tau(\text{C}^\alpha, \text{C}', \text{N})$	114.0°
$\text{C}'-\text{O}$	1.24	$\tau(\text{N}, \text{C}^\alpha, \text{C}^\beta)$	109.5°	$\tau(\text{C}^\alpha, \text{C}', \text{O})$	121.0°
$\text{C}'-\text{N}$	1.32	$\tau(\text{N}, \text{C}^\alpha, \text{H}^\alpha)$	112.5°	$\tau(\text{O}, \text{C}', \text{N})$	125.0°
$\text{N}-\text{C}^\alpha$	1.47	$\tau(\text{H}^\alpha, \text{C}^\alpha, \text{C}')$	106.3°	$\tau(\text{C}', \text{N}, \text{H})$	123.0°
$\text{C}^\alpha-\text{C}^\beta$	1.54	$\tau(\text{H}^\alpha, \text{C}^\alpha, \text{C}^\beta)$	107.9°	$\tau(\text{C}', \text{N}, \text{C}^\alpha)$	125.0°
$\text{C}^\alpha-\text{H}$	1.00	$\tau(\text{C}^\beta, \text{C}^\alpha, \text{C}')$	107.9°	$\tau(\text{H}, \text{N}, \text{C}^\alpha)$	114.0°
$\text{C}^\beta-\text{H}$	1.00				

Table III. Partial charges on each atom

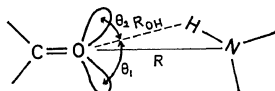
Atom	Charge, in units of e , the electronic charge	Atom	Charge, in units of e , the electronic charge
C $^{\alpha}$	+0.046	H	+0.204
C'	+0.318	C $^{\beta}$	-0.110
O	-0.422	H $^{\beta}$	+0.040
N	-0.202	H $^{\alpha}$	+0.046

The electrostatic interactions are calculated with the monopole approximation. The partial charges on each atom were taken from the results of Poland, *et al.*,⁸ and they are shown in Table III. These partial charges are slightly different from those of Ooi, *et al.*,⁴ but they were obtained in a systematic way for all amino acids and thus are useful for proteins as well. We take 4 as the dielectric constant.

Hydrogen-bonding interaction between the NH and CO groups of the backbone chain is represented by the potential function of Lippincott and Schroeder,⁹ modified by Ooi, *et al.*⁴ The final equation is

$$U_{\text{HB}} = Ae^{-bR} - \left(\frac{1}{2}A\right)\left(\frac{R_0}{R}\right)^6 \exp(-bR_0) - D^* \left\{ \left[\frac{1+(6)^{1/2} \cos \theta_1}{1+(6)^{1/2}} \right]^2 + \left[\frac{1+(6)^{1/2} \cos \theta_2}{1+(6)^{1/2}} \right]^2 \right\} \exp \left[\frac{-n^* R_{\text{OH}}^2}{2(R-r)} \right] \quad (1)$$

where R and R_{OH} are N—O and O—H distances respectively, and θ_1 and θ_2 are the angles made between HO and the two directions of lone pair orbitals of the O atom, and their geometrical relation is shown in Figure 1. Ooi, *et al.*,⁴ dealt with R_{OH} as constant, but we considered R_{OH} as variable. The values of parameters are as follows: $A=4.941 \times 10^6$, $b=4.8$, $D^*=82.4$, $n^*=13.15$, $r=1.01 \text{ \AA}$, $R_0=2.85 \text{ \AA}$. They are obtained from ref 4. If the geometrical arrangement of the CO and NH groups is unfavorable for forming a hydrogen bond,

**Figure 1.** Geometrical relation of hydrogen-bonding atoms.

the nonbonded and electrostatic interactions between the O atom in the CO group and N and H atoms in the NH group should be taken into consideration instead of the function (1) mentioned above. On the other hand if the arrangement is favorable, we have to use eq 1 and the nonbonded and electrostatic interactions should be deleted, since these interactions are taken into account in eq 1. The criteria for the favorable arrangement are described in ref 4.

The calculation of the interaction energies was simplified by the regularity condition, *i.e.*, the set of φ , ψ , and χ is the same in every residue.

The total energy of a conformation of poly(L-alanine), as well as its structure of interactions was calculated on a computer. By the structure of interaction we mean the way in which the energies are distributed into the pairs of peptide units. This structure of interaction is essential to our discussion.

RESULTS AND DISCUSSION

Results obtained are summarized in Table I and in Figures 2–5. The minimum of energy lies at $\varphi=124.5^\circ$, $\psi=128.1^\circ$, and $\chi=180.8^\circ$. In Table I the contributions of each type of interaction to the total energy at the α_{R} -helix are given. Figures 2 and 3 show the energy contour diagrams of poly(L-alanine) with and without hydrogen-bonding energy respectively in the region near the α_{R} -helix by assuming that χ is always kept to the value 180.8° . One sees that without hydrogen bonding the energy is still minimum near the α_{R} -conformation, but it becomes shallower. These results are essentially same as those of Ooi, *et al.*,⁴ but the slight difference comes from the differences of the angle $\tau(\text{N}-\text{C}^{\alpha}-\text{C}')$, of the potential function of the hydrogen bonding and of the treatment of electrostatic interaction when the hydrogen bond is formed. Figures 4 and 5 show the structures of interaction in the cases of α_{R} -conformation and the conformation slightly deformed ($\Delta\varphi=-2.5^\circ$, $\Delta\psi=-2.4^\circ$) from α_{R} respectively. The characteristic feature of Figure 4 is the presence of an intensive interaction between the 1st and 4th units (1–4 interaction) which arises mainly from the hy-

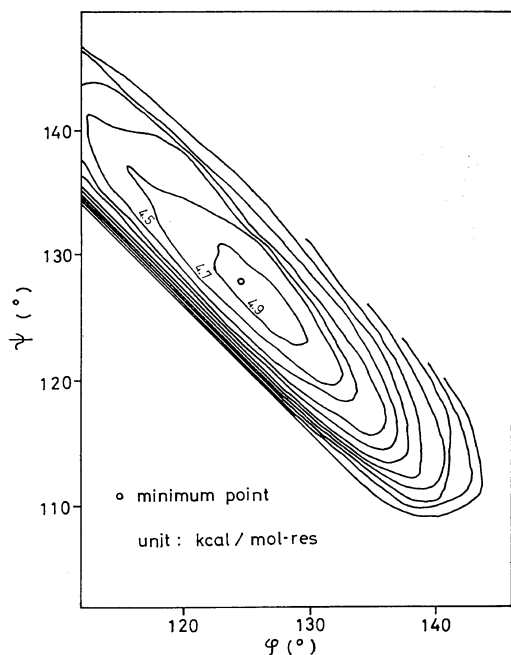


Figure 2. Energy contour diagram in the region near the α_R -helix.

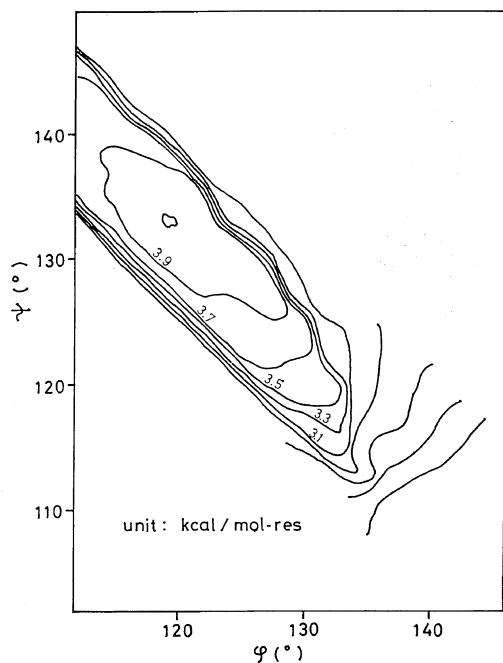


Figure 3. Energy contour diagram without the hydrogen-bonding interaction in the region near the α_R -helix.

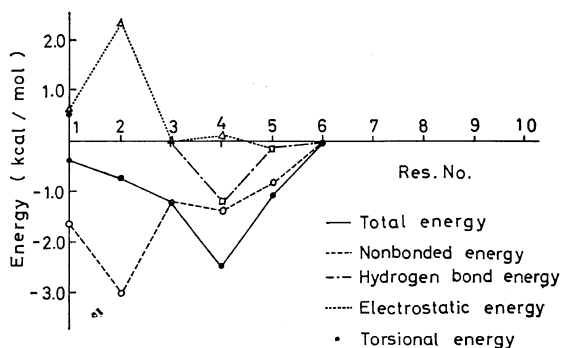


Figure 4. The structure of interaction energy of the α_R -helix.

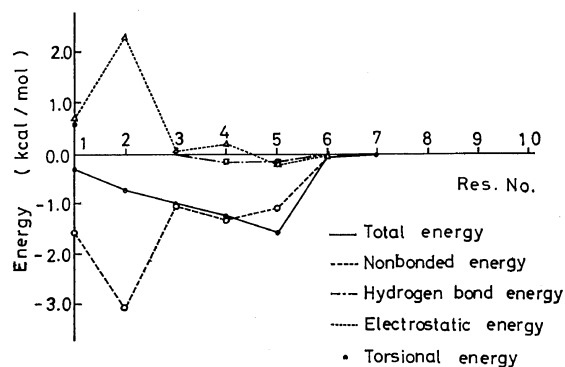


Figure 5. The structure of interaction energy of the conformation slightly deformed from the α_R -helix.

drogen bonding. In Figure 5, on the contrary, this hydrogen bonding and thus 1—4 interaction disappear, while the other kinds of energies remain unchanged. The 1—4 interaction is active only in a small region around the α -helical conformation. This fact manifests itself in the smallness of parameters σ in Zimm-Bragg theory² and v in Lifson—Roig theory.³ The spatially specific hydrogen bonding fastens the peptide chain through the 1—4 interaction.

In addition, the electrostatic interaction between nearest neighbor units is found to be positive and large by virtue of the nearly head-to-head arrangement of dipoles and cancels the nonbonded interaction. Therefore the nearest neighbor interaction is not necessarily favorable for the α -helix. Thus one sees that for the nucleation of a helix an unfavorable conformation of small entropy and energy is

required, but once this conformation is achieved, the helical conformation becomes stable. This is in analogy with a one-dimensional lattice gas undergoing diffuse phase transition with repulsive interaction between nearest neighbors and attractive interaction between second neighbors.¹⁰ Thus we can understand the reason that the Zimm—Bragg or Lifson—Roig theory, which takes only the hydrogen-bonding interaction, can yield the relations which can be adequately reproduced within the experiments. The stability of the tertiary structure of proteins can be treated similarly through the structures of interaction energy. This will be discussed in a separate paper.

Acknowledgments. We would like to express our thanks to Professor Ooi for his discussions on various aspects of the present paper. This work was supported partly by a research grant from Institute of Fundamental Physics, Kyoto University.

REFERENCES

1. A. Miyake, *J. Polym. Sci.*, **46**, 169 (1960).
2. B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **34**, 526 (1959).
3. S. Lifson and A. Roig, *J. Chem. Phys.*, **34**, 1963 (1961).
4. T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *J. Chem. Phys.*, **46**, 4410 (1967).
5. J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Némethy, G. N. Ramachandran, and H. A. Scheraga, *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966); *Biopolymers*, **4**, 121 (1966).
6. Data given by the courtesies of Drs. Kendrew and Phillips.
7. K. Nishikawa, T. Ooi, Y. Isogai, and N. Saitô, *J. Phys. Soc. Japan*, **32**, 1331 (1972).
8. D. Poland and H. A. Scheraga, *Biochemistry*, **6**, 3791 (1967).
9. E. R. Lippincott and R. Schroeder, *J. Chem. Phys.*, **23**, 1099 (1955).
10. N. Saitô and M. Oonuki, *J. Phys. Soc. Japan*, **29**, 69 (1970).