

Collagen의 수화에 대한 이론적 연구

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Theoretical Study of the Hydration of Collagen

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요 약. Collagen의 model compound인 poly(Gly-Pro-Pro)의 수화에 관해서 empirical potential energy 함수를 사용하여 이론적으로 연구하였다.

물 분자와 model compound 간의 상호작용 energy를 최소화시켜서 물 분자들의 위치와 결합 energies를 계산하였다. 다음에는 일차수화층에 물이 붙음으로써 생기는 안정화 energy를 계산하고, 전체 안정화에 기여하는 여러 energy components의 기여도에 대해 논의하였다.

ABSTRACT. A theoretical study of the hydration of the model compound of collagen, poly(Gly-Pro-Pro), has been carried out using empirical potential energy functions.

The optimum locations and binding energies of water molecules bound to the model compound have been determined by minimizing the interaction energy.

The stabilization energy due to the presence of water in the first hydration shell has been evaluated by comparing the internal interaction energies between the different groups of the model compound in its non-hydrated and hydrated states.

The different energy components contributing to the overall stabilization are determined and discussed.

INTRODUCTION

Collagen is the major protein constituent of our body and it has a triple-stranded helix structured which is different from α -helix or β -pleate sheet structures of other proteins.

The first triple-stranded helix model for collagen was that suggested by Ramachandran and Kartha^{1,2}. This was consisted of three left-handed helical chains joined by interchain hydrogen bonds, by the introduction of a right-

handed rope twist so that the individual chains had a coiled-coil conformation.

It is well known that water molecules perform a important role to maintain the triple helical structure of collagen.

The hydration of collagen is thoroughly studied by various experimental methods such as X-ray diffraction patterns^{3,4}, NMR⁵⁻⁷, IR⁸, dielectric constant⁹ and thermodynamic properties¹⁰ measurements.

Recently several two-state models for water

bound to collagen have been proposed on the basis of wide line NMR spectra obtained with H₂O and D₂O: in one state, water molecules are tightly bound to collagen and in the other state, they are freely rotating.

From the theoretical point of view, a refined quantum mechanical study has been performed recently, using *ab-initio* SCF methods.

This study enabled a more precise location of the water molecules on these substrates and determination of the energies of interaction involved, of the structure and lability of the bound water.

These theoretical calculations which are restricted to small molecules, can be extended to macromolecules by using empirical potential energy functions.

Very recently, Perahia, Jhon and Pullman¹¹ have determined the number and locations of water molecules bound to B-DNA and calculated the binding energies of them and the stabilization due to hydration using the empirical potential energy functions.

The aim of present research is to determine the hydration scheme of collagen and evaluate the stabilizing effect of the water molecules in triple-stranded helix structure of collagen using the same potential energy functions as used by Perahia *et al.*¹¹.

PROCEDURE

1. The Method

The basic formulae and the parametrization used for the potential energy functions follow the general scheme presented by Caillet and Claverie^{12,13}. The interaction energy is composed of three long-range contributions (electrostatic, polarization and dispersion) and a short-range repulsive contribution.

For hydrogen bonding, new parameters in the repulsive term at short distances are used.

The different components are computed as follows.

Electrostatic Energy. Each molecule is considered as a set of atomic net charges (the so-called "monopoles"), hence, the electrostatic interaction energy is given by

$$E_{el}(1, 2) = 332.072 \sum_i^{(1)} \sum_j^{(2)} \frac{q_i q_j}{R_{ij}} \text{ (kcal/mole)} \quad (1)$$

where the summations $\sum_i^{(1)}$ and $\sum_j^{(2)}$ run over the atoms of molecules 1 and 2, respectively and R_{ij} is the distance (in Å) between atoms i and j ; q_i and q_j are the net charges (in electron unit) of atoms i and j of molecules 1 and 2.

The net atomic charges of the polypeptide chains are taken from the result of Poland and Scheraga¹⁴.

Polarization Energy. The polarization energy of say molecule 1 is evaluated as the sum of atom polarization contributions:

$$E_{pol}(1) = -\frac{1}{2} \times 3320.72 \sum_i \alpha_i (\vec{\epsilon}_i)^2 \text{ (kcal/mole)} \quad (2)$$

where $\vec{\epsilon}_i$ is the electric field created at atom i of molecule 1 by molecule 2, and α_i is the mean polarizability attributed to atom i , which is obtained from bond polarizability¹⁵.

Dispersion and Repulsion Energies. The formula for both the dispersion and repulsion energies is written as

$$E_{disp} + E_{rep} = \sum_i^{(1)} \sum_j^{(2)} \epsilon_{(i,j)} \quad (3)$$

where each atom-atom contribution $\epsilon_{(i,j)}$ is the sum of a dispersion term and a repulsion term:

$$\epsilon_{(i,j)} = k_i k_j \left[-\frac{A}{Z^6} + \left(1 - \frac{q_i}{N_i^{val}}\right) \left(1 - \frac{q_j}{N_j^{val}}\right) \cdot C \cdot e^{-\alpha Z} \right] \quad (4)$$

where

$$Z = R_{ij} / R_{ij}^0 \quad (5)$$

and

$$R_{ij}^0 = \sqrt{(2R_i^w)(2R_j^w)} \quad (6)$$

R_i^w and R_j^w are the Van der Waals radii of atoms i and j . $R_H^w=1.2\text{Å}$, $R_C^w=1.7\text{Å}$, $R_N^w=1.6\text{Å}$ and $R_O^w=1.5\text{Å}$ are taken from Bondi¹⁶.

The factors $(1-q_i/N_i^{val})$ represent the influence of the atomic electrons for the neutral atom.

The multiplicative factors k_i and k_j allow for the variation of the minimum value of the potential $\varepsilon_{(i,j)}$ according to the nature of the interacting atoms ($k_H=k_C=1$, $k_N=1.18$, $k_O=1.36$).

Finally, the following values are used for A, C and α ; $A=0.214\text{ kcal/mole}$, $C=4.7 \times 10^4\text{ kcal/mole}$, $\alpha=12.35$.

The Case of Hydrogen Bond. The following procedure is adopted; first, the distances are divided into two types, R_m and R_M (with $R_m < R_M$); if $R > R_M$, the normal parameters are used for A, C, α ; if $R < R_m$ the modified parameters A', C', α' (with $A' < A$, $C' < C$, $\alpha' > \alpha$) are used; and for $R_m < R < R_M$, we use interpolated values of these parameters according to the formula¹²:

$$K(x) = \frac{K+K'}{2} + \frac{K-K'}{2} (0.375x^5 - 1.25x^3 + 1.875x) \quad (7)$$

where K represents one of the symbols A, C or α and

$$x = \frac{R - \frac{1}{2}(R_M + R_m)}{\frac{1}{2}(R_M - R_m)}$$

The values $R_m=1.8\text{Å}$, $R_M=2.6\text{Å}$, $A'=A/5$, $C'=C/27$ and $\alpha'=13.8$ are taken¹⁷.

2. Model Compound

The synthetic polypeptide, $(\text{Gly-Pro-Pro})_n$, was chosen as the model compound of collagen.

It was consisted of a triple-stranded helix, with three amino acid residues in each strand which is presented in Fig. 1.

The amino acid residues are designated successively be $G_A, P1_A, P2_A$, etc., as seen in Fig. 1 and 2; G stands for glycine, $P1$ and $P2$ represent the proline residues following the glycine residue, respectively, and the subscripts A, B, C indicate the chains to which the residue belongs.

Each atom is designated by its notation in the residue (see Fig. 1) followed by the designation, within parenthesis, of the residue to which it belongs.

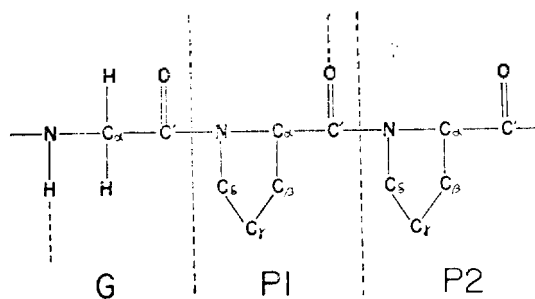


Fig. 1 Structural formula for one tripeptide unit of the model compound with the notations.

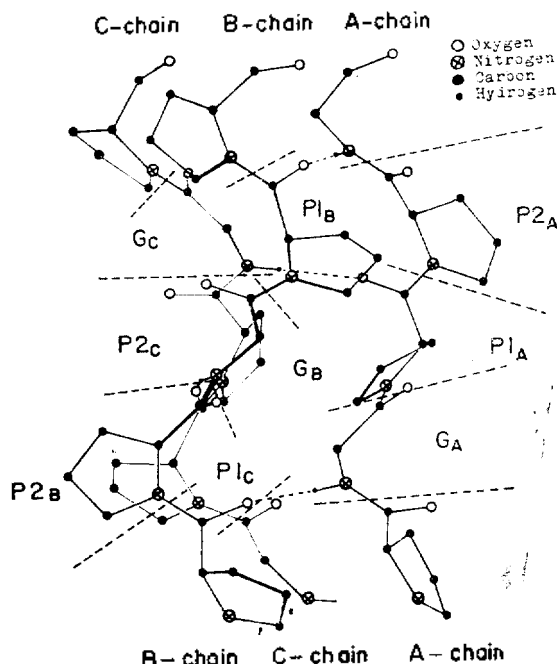


Fig. 2. Projection along the helical axis of the model compound.

The cylindrical coordinates of the atoms in chain A are taken from Yonath and Traub⁴.

3. Determination of the Hydration Scheme of the Model Compound

The hydration scheme of the triple-helix model compound has been studied explicitly. First of all, attention was centered on the peptide group which is *a priori* hydrophilic.

To determine the geometrical position of a water molecule with respect to the model compound, six parameters, d_1 , θ_1 , θ_2 , φ_1 , φ_2 , φ_3 depicted in Fig. 3, are needed. If A_1 , A_2 and A_3 are three successive atoms on the model compound with known coordinates and with A_1 forming a hydrogen bond with the water molecule, d_1 is the A_1H distance, θ_1 and θ_2 are the A_2A_1H and A_1HO angles, respectively, and φ_1 , φ_2 and φ_3 are the angles formed by the pairs of planes $A_3A_2A_1$ and A_2A_1H , A_2A_1H and A_1HO , A_1HO and HOH' , respectively. The dihedral angles φ_1 , φ_2 and φ_3 obey the following conventions (see Fig. 4): (i) the zero values correspond to a *cis* planar position of the external bonds adjacent to the rotating bond and (ii) the positive direction corresponds to a right-

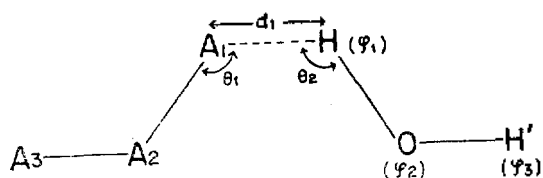


Fig. 3. Six variables defining the positions of a water molecule.

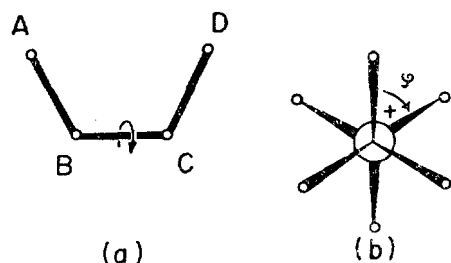


Fig. 4. Conventions used for dihedral angles. (a) Zero value for the dihedral angle about the bond. (b) positive values of the dihedral angles.

handed rotation.

The cartesian coordinates of the water molecule are computed as a function of six parameters, d_1 , θ_1 , θ_2 , φ_1 , φ_2 and φ_3 , in the reference system in which A_1 , A_2 and A_3 are defined. The method developed by Thompson^{18,19} is used for such computations.

In the first step of the calculations, the potential energy maps have been determined as a function of two variables, φ_1 and φ_3 , for different values of d_1 , θ_1 , θ_2 and φ_2 . The distance, d_1 , has been varied in the range of possible hydrogen bonding formation. The dihedral angles have been varied from 0° to 360° by 36° increments. The θ_1 and θ_2 angles have been varied from 90° to 180° with the same increments. These maps have been determined for all possible hydration sites; the interaction energy computed for a given water molecule includes the pair-wise interactions of the atoms of this water with the atoms of the model compound and the atoms of the other water molecules which has already been computed and located in their preferential positions. The calculations, have thus been performed by locating successively each water molecule until no more such water molecules can be located for a direct binding to the model compound.

In the second step of the calculations, we have optimized the locations found in the preceding study by minimizing the interaction energy of each water molecule through the variation of the six parameters (d_1 , θ_1 , θ_2 , φ_1 , φ_2 , and φ_3) simultaneously.

We used the quasi-Newton method developed by Fletcher²⁰ in the minimization of the interaction energy.

4. Evaluation of the Stabilization Energy due to Hydration

We divide the model compound into nine amino acid residues as seen in Fig. 2.

The stabilization energy due to hydration has been evaluated by comparing the internal interaction energies between the different groups of the model compound in its non-hydrated and hydrated states. The hydrated model compound is considered as a "supermolecule", a unique entity formed by the substrate skeleton plus water molecules of the first hydration shell. The binding energies of the water molecules to the hydrophilic sites are not included (water being simply a part of the supermolecule), although they may, of course, always be added to the balance. In cases in which water molecules are involved in two hydrogen bonds, they were counted with the subunit to which the binding was the strongest.

Such a procedure enables a direct evaluation of the amount of stabilization of the model compound due to the presence of the bound water molecules.

RESULTS AND DISCUSSION

1. Hydration of the Collagen Model Compound

The results showing the optimum positions and binding energies of the bound water molecules are listed in *Table 1*.

Three water molecules per tripeptide are attached to the model compound: one of them, *W1*, is attached to the carbonyl groups of *P2* and *P1* of the same chain (chain *B*), the other one, *W2*, is bridged between the oxygen atoms of previously attached water molecule (*W1*) and the carbonyl group of *G* of different chain

(chain *A*), and the remaining one, *W3*, is attached to the carbonyl groups of *G* and *P2* of the same chain (chain *A*).

The binding energies of these water molecules, *W1*, *W2* and *W3*, are -15.7, -11.1 and -8.9 kcal/mole, respectively.

These binding energies are comparable to those of the water molecules bound to bases and sugars in B-DNA (from -9 to -16 kcal/mole)¹¹. The present results concerning the hydration of the peptide group are in agreement with the theoretical *ab-initio* SCF computations on the hydration scheme of formamide dimer carried out by Port and Pullman²¹ and with the empirical energy calculations on poly-proline II by Krimm and Venkatachalam²² and on dialanine by Forsythe and Hopfinger²³.

The high binding energy found for *W1* in our calculations stems from the fact that this water molecule forms also another hydrogen bond with *W2*.

The optimum positions of the other six water molecules are obtained from the successive transformation of the positions of these three water molecules, as was expected from the structure of the model compound (see *Fig. 2*).

We have indicated the projection along the helical axis of the model compound including the bound water molecules in *Fig. 5*. The water molecules are positioned along the grooves of the model compound as seen in *Fig. 5*.

The hydrogen bond distances for water molecules sharing two or three sites are indicated in *Table 2*.

Table 1. Locations and the binding energies of the hydrated water molecules in the model compound of collagen.

Hydrated Water	Positions of hydrated water						Reference atoms			Binding energies - ΔE (kcal/mole)
	d_1 (Å)	θ_1 (deg)	θ_2 (deg)	φ_1 (deg)	φ_2 (deg)	φ_3 (deg)	A_1	A_2	A_3	
<i>W1</i>	1.97	120.9	130.8	123.3	303.4	336.4	O(<i>P2_B</i>)	C'(<i>P2_B</i>)	C(<i>P2_B</i>)	15.7
<i>W2</i>	1.87	143.5	172.1	5.1	339.7	350.7	O(<i>G_A</i>)	C'(<i>G_A</i>)	C(<i>G_A</i>)	11.1
<i>W3</i>	1.99	113.1	141.2	114.8	351.9	306.2	O(<i>G_A</i>)	C'(<i>G_A</i>)	C(<i>G_A</i>)	8.9

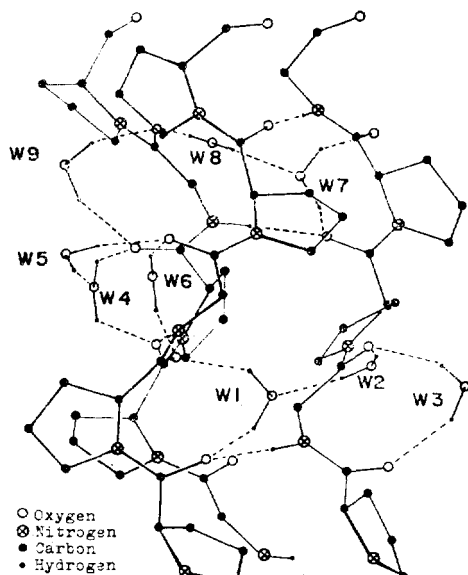


Fig. 5. Projection along the helical axis of the model compound indicating the positions of the water molecules attached.

Table 2. Hydrogen bond distances for the water molecules sharing two or three sites.

Water molecules	Hydrogen bonds	Distances (Å)
W4	O(P2C).....H(W4)	1.97
	O(P1C).....H'(W4)	1.91
	O(W4).....H'(W5)	1.84
W5	O(G _B).....H(W5)	1.87
	O(W4).....H'(W5)	1.84
W6	O(G _B).....H(W6)	1.99
	O(P2B).....H'(W6)	1.93

Yonath and Traub⁴ already determined the possible positions for two water molecules in our model compound in comparison of the calculated and observed intensities of X-ray diffraction pattern. In their results, one water molecule makes hydrogen bonds to O(G_B) and N(G_B), the other to O(P1C) and O(P2C), and they are also hydrogen bonded to each other. But this is impossible because additional hydrogen atom needed to make a hydrogen bond each other,

does not exist. (The four hydrogen atoms of the water molecules are donated to make four hydrogen bonds. The NH(G_B) group makes a interchain hydrogen bond with O(P1C), hence, the hydrogen atom of NH(G_B) cannot participate in the hydrogen bond formation with water molecules.)

Moreover, our calculation showed that the hydrogen bond between O(G_B) and O(P2B) through water molecule is energetically more favorable than that between O(G_B) and N(G_B). From the above facts, our results are more reasonable than those of Yonath and Traub.

2. Stabilization of the Collagen Model Compound due to Hydration.

We have divided the three water molecules in each chain as follows; two of them belong to the glycine residue, G, the remaining one to the proline residue, P2, which precedes the glycine residue, and no water molecule to P1 following G.

The interaction energies between the different groups in the collagen model compound both in their hydrated and non-hydrated states are presented in Table 3. The right-handed triangle of the table concerns the hydrated state and the left-handed one the non-hydrated state.

It is seen that the total interaction energy between these groups in their hydrated state is -87 kcal/mole as compared to -44 kcal/mole in their non-hydrated state.

The stabilization energy due to the hydration of the collagen model compound is of the order of 43 kcal/mole. It is surprising that this stabilization energy of collagen is slightly different from that of B-DNA (55 kcal/mole)¹¹, while the binding energies of water molecules of collagen are much less than those of B-DNA. Of course, the precise amount of this stabilization energy may be questionable. What is certainly significant, however, is its

Table 3. Total interaction energies (kcal/mole) between the different groups of the model compound in their hydrated and non-hydrated states.

	G_A	$P1_A$	$P2_A$	$P2_B$	G_B	$P1_B$	$P1_C$	$P2_C$	G_C	
G_A		-3.0	-2.7	-4.6	-0.4	-2.1	-1.9	-0.1	0.1	Hydrated state
$P1_A$	-1.6		-2.6	-0.4	-1.8	-0.2	-0.2	0.0	-6.2	
$P2_A$	-1.9	0.5		-0.6	-0.1	0.1	-0.3	-2.2	-4.5	
$P2_B$	-0.1	-0.2	-0.3		-13.9	-1.8	0.1	-2.3	-0.3	
G_B	-1.4	-1.5	-0.2	-8.4		-2.9	-6.2	-4.6	-0.4	
$P1_B$	-1.3	-0.2	0.3	-1.1	-1.5		-0.1	-0.4	-1.8	
$P1_C$	-1.5	-0.2	-0.2	0.2	-4.8	-0.1		-2.6	-1.7	
$P2_C$	-0.2	0.2	-0.5	-0.5	-0.1	-0.2	0.5		-13.9	
G_C	-0.2	-4.8	-0.1	-0.3	-1.4	-1.5	-1.0	-8.4		
Total	-43.9	Non-hydrated state								

Table 4. Electrostatic energy component of interaction energies.

	G_A	$P1_A$	$P2_A$	$P2_B$	G_B	$P1_B$	$P1_C$	$P2_C$	G_C	Total
G_A		-2.1	-1.0	-3.0	1.5	-0.3	-1.0	0.4	0.3	Hydrated state
$P1_A$	-1.7		-2.4	-0.2	-0.9	0.6	0.6	-1.0	-4.9	
$P2_A$	-0.9	0.5		-0.5	0.4	-1.0	-0.2	-1.0	-3.0	
$P2_B$	0.6	-0.1	-0.2		-11.0	-0.7	-1.0	-1.0	-0.1	
G_B	0.1	-0.6	0.2	-7.6		-2.1	-4.8	-3.1	1.5	
$P1_B$	-0.4	0.6	-0.9	-0.6	-1.7		0.0	-0.2	-0.9	
$P1_C$	-0.6	0.6	-0.1	-0.9	-3.8	0.0		-2.4	-1.1	
$P2_C$	0.2	-0.9	-0.3	-0.3	0.6	-0.1	0.5		-11.0	
G_C	-0.1	-3.8	0.6	-0.1	0.1	-0.6	-0.5	-7.6		
Total	-29.9	Non-hydrated state								

relatively large value and perhaps still more is the information which it may produce on the nature of the forces involved in the stabilization and their relative contribution.

Table 4 shows that the stabilization through hydration of collagen is mainly brought about by the increase of the attractive interaction between some groups due to the introduction of water molecules into the system.

The major contribution to the stabilization produced by hydration is the increase of the attractive interaction between glycines (G) and prolines ($P2$) which precede the glycine residue. The effect amounts to about 5~6 kcal/mole. In non-hydrated state, the interaction energy

between G and $P1$ residues of different chains, is relatively large, which is due to the interchain hydrogen bond.

Table 4~6 present the contributions of the different components of the empirical potential energy function to this overall stabilization.

The electrostatic energy component which amounts to about 27 kcal/mole represents the major contribution to the stabilization, the polarization term contributes to about 6 kcal/mole, and the dispersion and repulsion term about 10 kcal/mole.

The increase of the attraction between G and $P2$ also performs the major role in the stabilization due to hydration in each component.

Table 5. Polarization energy component of interaction energies.

	G_A	$P1_A$	$P2_A$	$P2_B$	G_B	$P1_B$	$P1_C$	$P2_C$	G_C	Total
G_A		-1.0	-0.1	-1.2	-0.3	-0.2	-0.1	-0.1	-0.0	-16.9
$P1_A$	-1.0		-1.5	-0.0	-0.1	-0.1	-0.1	-0.3	-0.9	Hydrated state
$P2_A$	-0.2	-1.2		-0.0	-0.1	-0.3	-0.0	-0.2	-1.2	
$P2_B$	-0.1	-0.0	-0.0		-1.6	-0.1	-0.3	-0.2	-0.0	
G_B	-0.2	-0.1	-0.0	-1.3		-1.0	-0.9	-1.2	-0.3	
$P1_B$	-0.2	-0.1	-0.3	-0.1	-1.0		-0.0	-0.0	-0.1	
$P1_C$	-0.1	-0.1	-0.0	-0.3	-0.8	-0.0		-1.5	-0.1	
$P2_C$	-0.0	-0.3	-0.0	-0.0	-0.1	-0.0	-1.2		-1.6	
G_C	-0.0	-0.8	-0.1	-0.0	-0.2	-0.1	-0.1	-1.3		
Total	-11.2	Nonhydrated state								

Table 6. Dispersion and repulsion energy component of interaction energies.

	G_A	$P1_A$	$P2_A$	$P2_B$	G_B	$P1_B$	$P1_C$	$P2_C$	G_C	Total
G_A		0.2	-1.5	-0.3	-1.6	-1.6	-0.8	-0.4	-0.2	-12.9
$P1_A$	1.2		1.3	-0.2	-0.8	-0.7	-0.7	1.4	-0.4	Hydrated state
$P2_A$	-0.7	1.3		0.1	-0.4	1.5	-0.1	-1.1	-0.4	
$P2_B$	-0.7	-0.1	-0.0		-1.3	-1.1	1.4	-1.1	-0.2	
G_B	-1.3	-0.7	-0.3	0.4		0.2	-0.4	-0.3	-1.6	
$P1_B$	-0.7	-0.7	1.5	-0.5	1.2		-0.1	-0.2	-0.8	
$P1_C$	-0.7	-0.7	-0.0	1.4	-0.3	-0.1		1.2	-0.5	
$P2_C$	-0.3	1.4	-0.2	-0.2	-0.7	-0.1	1.2		-1.3	
G_C	-0.1	-0.3	-0.7	-0.1	-1.3	-0.7	-0.4	0.4		
Total	-2.8	Nonhydrated state								

For example, the stabilizing effect produced by the hydration for the interactions between G and $P2$ of the different chains is due to the strong increase (3.6 kcal/mole) of the attractive electrostatic energy which becomes attractive (-3.0 kcal/mole) from repulsive (0.6 kcal/mole), and to a smaller increase of polarization energy (1.1 kcal/mole) and the dispersion and repulsion term represents the slight destabilizing effects (0.4 kcal/mole).

In this case, the strong electrostatic repulsion between the carbonyl groups of G and $P2$ is highly reduced by the presence of water molecules.

In the case of G and $P2$ interaction in the same chain, hydration brings about the stabiliza-

tion of the structure by 3.4 kcal/mole through electrostatic effect, 1.7 kcal/mole through the dispersion and repulsion term, and 0.3 kcal/mole through the polarization effect with the resultant overall stabilization of about 5.5 kcal/mole.

Very recently, Engel *et al.*²⁴ have insisted that the presence of water molecules is not an essential part of the triple-helical structure formed by either $(\text{Gly-Pro-Pro})_n$ or $(\text{Gly-Pro-Hyp})_n$ from the fact that the triple helical structure is maintained in spite of the absence of water molecules.

This can be qualitatively explained by the fact that the difference between the minimum potential energies of the parallel-chain complex of $(\text{Gly-Pro-Pro})_4$ (-68.1 kcal/mole) and of

the triple-stranded coiled-coil complex of same molecule (-112.8 kcal/mole), is about -45 kcal/mole, which indicates that the triple helix structure is more favorable than the single chain structure²⁵.

However, it is obvious that water molecules stabilize the triple helix structure through the additional interchain hydrogen bond in aqueous environment.

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