

Modeling of Folding and Unfolding Mechanisms in Alanine-Based α -Helical Polypeptides

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α -Helix formation is known to be opposed by the entropy loss due to the folding and favored by the energy of molecular interactions. However, the underlying mechanism of these factors is still being discussed. Here we have used the experimental and calculation data for short alanine-based peptides embedded in water to model the mechanism of helix folding and unfolding and to calculate microscopically the free energy factors of alanine in the frame of helix coil conformational integrals. Classical helix–coil transition theories take into account the interactions in a peptide chain only if the $i, i + 3$ peptide bond participates in hydrogen bonding. But quantum mechanical calculations showed that interactions of the $i, i + 2$ peptide bond play an important role in helix folding too. We also included the short-range repulsive interactions due to molecular steric clashes and the end effects due to polar/hydrogen-bonding interactions at the N and C termini. The helix and coil regions of peptide conformational space were defined using an experimental steric criterion for hydrogen bonding. Arginine helix propensity was discussed and estimated. Monte Carlo numerical simulations of thermodynamics and kinetics for the 21 amino acid α -helical polypeptide Ac-A₅(AAARA)₃A-NMe were carried out and found to be in an agreement with the experimental results.

1. Introduction

It is well established in experiments that tertiary structures of a protein's native folds contain a substantial amount of α -helices (for example, see ref 1). α -Helices can be formed by short alanine-based peptide (AP) chains in an aqueous environment even without tertiary interactions,^{2,3} thus providing an opportunity to study the mechanism of folding in the systems that are simpler than the globular proteins. It is believed that an understanding of helix folding of AP peptides will be helpful for investigating the interplay between tertiary and secondary structures in protein folding.⁴ The repeating hydrogen bonding between CO and NH of i and $i + 3$ of a peptide bond, respectively, was the clue to find the steric structure of the α -helix.⁵ In classical Zimm–Bragg (ZB) and Lifson–Roig (LR) thermodynamic helix–coil transition theories,^{6,7} a peptide molecule is viewed as a linear chain of two-state units that interact only if they participate in hydrogen bonding. Attempts were made to reexamine the classical picture by adding the interactions of neighboring chain units⁸ and the end effects due to polar or/and hydrogen-bonding interactions.⁹

A two-state description of the chain unit is useful in studying folding kinetics too. Helix folding ruled by a zipper mechanism for establishing native contacts was proposed in a model¹⁰ where the classical approach was expanded to include the side-chain interactions and the variable helix propensities. A zipper model can be treated analytically to solve thermodynamics¹¹ or to estimate kinetics.¹⁰ The general approach for the master equation of protein folding was proposed in the frame of the generalized kinetic Ising (GKI) model.¹² The Hamiltonian of this model

includes both native and nonnative interactions, and this particular case can be viewed as the zipper model.¹² With a realistic microscopic model of the interactions with a heat bath, one can use the GKI model to calculate the transition rates and to solve the master equation, as was done by the authors within the mean field approximation.¹² Currently, the Brownian motion of a biomolecule on a free energy potential surface is viewed as a good model of interactions with a heat bath.¹³ The theoretical framework for transition rates in such systems was proposed in ref 14. In the case of a rough energy landscape a multistate description of chain units is required. An analogue of the Potts model was proposed in ref 15 for helix folding. For a general form of interactions, the fundamental properties of a discrete multistate model were investigated in ref 16 by applying the theory of spin glasses.

Because of the complex nature of peptide–solvent systems, the microscopic derivation of free energy factors is a difficult problem in the aforementioned theories. Traditionally these factors were introduced as parameters according to the explored phenomenological model with subsequent fitting of experimental results to verify the model. Simple two-parameter LR and ZB theories are very popular among experimentalists and are used to treat the results of all-atom force field molecular dynamics (MD/FF) simulations^{17,18} too. However, the underlying physical picture of helix folding is still being discussed. Recently, for AP–water systems, substantial progress was achieved both in experiments and in microscopic calculations, thus giving more details about the issue.

Direct measurements of the enthalpy of helix elongation were done for alanine¹⁹ and for some other amino acids²⁰ using metal binding to induce helix formation. From these experiments, the enthalpy change of alanine does not depend on polypeptide length and is -0.9 ± 0.1 kcal/mol. On the contrary, the high-level quantum mechanical (QM) calculations^{21,22} for alanine

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dipeptide in gas phase showed an increase of the energy by ~ 4.5 kcal/mol for the transition between the coil and the helix states. The energy increase is mainly because of the unfavorable positions of the charges in the nearest peptide bond and must be covered by other interactions. Water plays a substantial role,^{22,23} tending to compensate for the Coulomb interactions. For long-range distances, the sum of the Coulomb interactions of the backbone polar groups is favorable to helix folding.²² Another important contribution can be due to the changes of the van der Waals interactions under transition from the coil to spatially compact α -helix.^{24,25} The question about the contribution of hydrogen bonding to the energy of helix elongation is still under discussion.⁴ The characteristic energy of water–peptide or backbone–backbone hydrogen bonding is 10-fold greater than $k_B T$ (k_B is the Boltzmann constant, T is the absolute temperature), and therefore with high probability these interactions are saturated both in the helix and in the coil conformations. QM calculations²⁶ give the energy as approximately -4.9 kcal/mol for hydrogen bonding between NH and CO polar groups of the backbone. A value of approximately -5.6 kcal/mol was reported in ref 27 for the hydrogen-bonding energy of water molecules. An energy of approximately -5.5 ± 0.5 kcal/mol was found in ref 28 for water–backbone hydrogen bonding using QM calculations while experimental data gave an energy of approximately -11.5 kcal/mol for the sum of the backbone NH and CO polar groups hydrogen-bonding with water.²³ Assuming that upon helix elongation two water–backbone hydrogen bonds are replaced by one water–water hydrogen bond and one hydrogen bond between the polar groups of the backbone, one can conclude that the balance of the energy contributions of hydrogen bonding to helix elongation is neutral or even unfavorable.

In the process of folding, the entropy changes could be favorable due to a hydrophobic effect or unfavorable due to freezing of the side chains and the backbone.²⁹ It was proposed to explain the differences in the helix propensities of amino acids by the differences in the entropy losses of their side chains.³⁰ Alanine has the highest helix propensity, and experimental data showed³¹ no entropy loss of the alanine side chain in folding. However, the helix propensities of other amino acids can be different also due to the energies of the side-chain interactions.^{20,32} Another important source of the entropy is the cost of binding of water molecules to the polar groups of the backbone.³³ The shielding of the backbone polar groups due to the water–side chain–backbone interactions is the possible source of helix stability in AP–water systems.^{17,34}

Modern theoretical studies of biological macromolecules are dominated by force-field-based approaches that provide powerful tools for simulations on microscopic scales. As for now, their results depend on the variants of the force fields.^{18,21,35,36} Also, even for relatively short peptides, statistically reliable results of MD/FF simulations at the moment are feasible only in unique worldwide computational projects.³⁷ Models with discrete descriptions of peptide chain units can include in a coarse-grained manner the results of precise QM calculations for small peptide–solvent systems and be useful for a qualitative understanding of helix folding as well as for quantitative analysis of experiments due to the cheap cost of numerical calculations. The aim of this article is to use a two-state description to model the mechanism of α -helix folding of AP peptides. In section 2, the conformational integrals proposed in the LR theory were used to derive the free energy potential of the helical peptide embedded in solvent. Section 3 is devoted to estimations of free energy parameters of alanine and arginine for the proposed

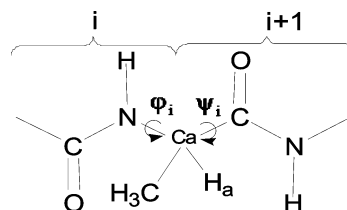


Figure 1. Peptide chain unit number i consisting of α carbon atom number i flanked by i and $i + 1$ peptide bonds

model. Monte Carlo (MC) simulations of thermodynamics and kinetics of α -helical Ac-A₅(AAARA)₃A-NME peptide (F_s peptide) are described in section 4. The next sections are discussions and conclusions.

2. Free Energy Potential of α -Helical Peptide Embedded in Solvent

It is convenient to view a polypeptide molecule as a chain of dipeptide units. The latter is a specific amino acid side chain and α carbon atom flanked by two planar groups of atoms usually referred as a peptide bond (Figure 1). The polypeptide chain of $N + 1$ peptide bond can be viewed as a chain of N dipeptide units. Neglecting the changes of bond lengths and angles, one can describe the conformational space Ω of the polypeptide by the set of torsional angles $\Omega_i = (\varphi_i, \psi_i, \dots)$

$$\Omega = \{\Omega_i\} \quad i = 1, \dots, N \quad (1)$$

where N is the number of dipeptide units. For alanine, the side-chain degrees of freedom are usually ignored, so a (φ, ψ) pair is enough to represent the conformational space of an alanine dipeptide unit. For a polypeptide embedded in solvent, one can find free energy potential $F^{(N)}$ as a function of Ω after averaging over the solvent coordinates^{14,38}

$$F^{(N)}(\Omega) = -\beta^{-1} \ln \frac{\int \delta(\Omega' - \Omega) \exp(-\beta E^{(N)}(\Omega', \Gamma^{(s)})) d\Omega' d\Gamma^{(s)}}{\int \exp(-\beta E^{(N)}(\Omega, \Gamma^{(s)})) d\Omega d\Gamma^{(s)}} \quad (2)$$

where $\Gamma^{(s)}$ are solvent coordinates of a polypeptide–solvent system, Ω' is the point in the conformational space of the polypeptide, and $E^{(N)}(\Omega', \Gamma^{(s)})$ is the energy of the system at the point $(\Omega', \Gamma^{(s)})$, $\beta = (k_B T)^{-1}$. The wealth of numerical calculations of $F^{(1)}(\Omega)$ potential^{35,36,39,40} for alanine dipeptide in water showed two distinctive potential wells that are divided by a free energy barrier. QM²² and MD/FF^{18,41} calculations of polyalanine–water systems also revealed the same picture for the dependence of $F^{(N)}(\Omega)$ on the Ω_i coordinate when all other coordinates are frozen. These results support the assumption of LR theory that Ω_i space can be reduced to the set of binary variables x_i

$$\mathbf{x} = \{x_i\} = \{x(\Omega_i)\} \quad i = 1, \dots, N \quad x_i \in (0, 1) \quad (3)$$

where $x(\Omega_i) = 1$ if Ω_i belongs to the potential well around the α -helix conformation and $x(\Omega_i) = 0$ if Ω_i belongs to the another potential well that is usually called a coil region. The position of the coil region can prefer the β or polyproline II regions⁴² depending on the solvent environment. Free energy potential as a function of \mathbf{x} is

$$F^{(N)}(\mathbf{x}) = -\beta^{-1} \ln \frac{\int \delta(\mathbf{x}'(\Omega) - \mathbf{x}) \exp(-\beta E^{(N)}(\Omega, \Gamma^{(s)})) d\Omega d\Gamma^{(s)}}{\int \exp(-\beta E^{(N)}(\Omega, \Gamma^{(s)})) d\Omega d\Gamma^{(s)}} \quad (4)$$

where $\mathbf{x}'(\Omega)$ is determined according to eq 3. Using eq 2, one can rewrite eq 4 as

$$F^{(N)}(\mathbf{x}) = -\beta^{-1} \ln \int \delta(\mathbf{x}'(\Omega) - \mathbf{x}) \exp(-\beta F^{(N)}(\Omega)) d\Omega \quad (5)$$

The $F^{(N)}(\Omega)$ function could be represented as the sum of the higher-order corrections to the $F_i^{(1)}(\Omega_i)$ potentials of each i th dipeptide unit

$$F^{(N)}(\Omega) = \sum_{i=1}^N J_i^{(1)}(\Omega_i) + \sum_{i=1}^{N-1} J_i^{(2)}(\Omega_i, \Omega_{i+1}) + \sum_{i=1}^{N-2} J_i^{(3)}(\Omega_i, \Omega_{i+1}, \Omega_{i+2}) + \dots + J^{(N)}(\Omega) \quad (6)$$

where the notation $J_i^{(1)}(\Omega_i) \equiv F_i^{(1)}(\Omega_i)$ is used for convenience. The LR theory⁷ takes into account only the $J_i^{(3)}$ term if three adjacent units are in the helix state $x_i x_{i+1} x_{i+2} = 1$

$$F^{(N)}(\Omega) \cong \sum_{i=1}^N J_i^{(1)}(\Omega_i) + \sum_{i=1}^{N-2} J_i^{(3)}(\Omega_i, \Omega_{i+1}, \Omega_{i+2}) x_i x_{i+1} x_{i+2} \quad (7)$$

The $J_i^{(3)}(\Omega_i, \Omega_{i+1}, \Omega_{i+2}) x_i x_{i+1} x_{i+2}$ contribution describes the interactions of the i , $i+1$, and $i+2$ units in a spatially compact α -helix structure. In essence, eq 7 is the short-range approximation of the $F^{(N)}(\Omega)$ potential in eq 6. From QM calculations,²² we found (section 3) that the nearest interactions of the i and $i+1$ units also play an important role in helix folding. The short-range steric clashes of i and $i+m$ units ($m > 1$) considerably decrease the available conformational space,^{43,44} and therefore the conformational entropy penalty on helix folding decreases too. Here we want to add these two short-range terms to the expansion in eq 7

$$F^{(N)}(\Omega) \cong \sum_{i=1}^N J_i^{(1)}(\Omega_i) + \sum_{i=1}^{N-1} J_i^{(2)}(\Omega_i, \Omega_{i+1}) x_i x_{i+1} + \sum_{i=1}^{N-2} J_i^{(3)}(\Omega_i, \Omega_{i+1}, \Omega_{i+2}) x_i x_{i+1} x_{i+2} + \tilde{J}^{(N)}(\Omega) \quad (8)$$

Similar to eq 7, $J_i^{(2)}$ contributes only if the i and $i+1$ units are in the helix state $x_i x_{i+1} = 1$. To estimate $F^{(N)}(\mathbf{x})$, we are going to use in eq 5 the zero terms of the $J_i^{(1)}$, $J_i^{(2)}$, and $J_i^{(3)}$ expansions over the appropriate minima Ω_i^1 and Ω_i^0 of the free energy potential. The $\tilde{J}^{(N)}(\Omega)$ potential due to short-range repulsions was approximated by the hard-sphere potential. With a constant, one can rewrite eq 8 as

$$F^{(N)}(\Omega) \cong \sum_{i=1}^N j_i^{(1)} x_i + \sum_{i=1}^{N-1} j_i^{(2)} x_i x_{i+1} + \sum_{i=1}^{N-2} j_i^{(3)} x_i x_{i+1} x_{i+2} + \tilde{j}^{(N)}(\Omega) + C \quad (9)$$

where

$$j_i^{(1)} = J_i^{(1)}(\Omega_i^1) - J_i^{(1)}(\Omega_i^0) \quad j_i^{(m)} = J_i^{(m)}(\Omega_i^1, \dots, \Omega_{i+m-1}^1) \quad m = 2, 3 \quad (10)$$

Substituting the potential function eq 9 into eq 5, one can obtain

$$F^{(N)}(\mathbf{x}) \cong \sum_{i=1}^N j_i^{(1)} x_i + \sum_{i=1}^{N-1} j_i^{(2)} x_i x_{i+1} + \sum_{i=1}^{N-2} j_i^{(3)} x_i x_{i+1} x_{i+2} - \beta^{-1} \ln \int \delta(\mathbf{x}'(\Omega) - \mathbf{x}) \tilde{j}^{(N)}(\Omega) d\Omega + C \quad (11)$$

where $\tilde{j}^{(N)}(\Omega) = \exp(-\beta \tilde{J}^{(N)}(\Omega))$. In accordance with previously reported results,⁴³ we found (section 3) that the $\tilde{j}^{(N)}(\Omega)$ potential excludes mainly the coil conformations. Assuming a uniform decrease of the number of coil conformations for each dipeptide unit, one can approximate $\tilde{j}^{(N)}(\Omega)$ as

$$\tilde{j}^{(N)}(\Omega) \cong \prod_{i=1}^N \gamma(x(\Omega_i)) \quad \gamma(0) < 1 \quad \gamma(1) = 1 \quad (12)$$

Using eq 12, one can transform the term with the integral in eq 11 to the sum

$$\beta^{-1} \ln \int \delta(\mathbf{x}'(\Omega) - \mathbf{x}) \tilde{j}^{(N)}(\Omega) d\Omega \cong k_B \sum_{i=1}^N \ln \int \delta(x'(\Omega_i) - x_i) \gamma(x_i) d\Omega_i = \sum_{i=1}^N (x_i - 1) \Delta S_i^c + C \quad (13)$$

where under the approximation in eq 9 ΔS_i^c describes the conformational entropy change of the i th unit. ΔS_i^c is normalized to be zero in the coil state

$$\Delta S_i^c = k_B \ln \frac{\int \delta(x'(\Omega_i) - x_i) \gamma(x_i) d\Omega_i}{\int \delta(x'(\Omega_i)) \gamma(0) d\Omega_i} \quad (14)$$

Substituting eq 13 into eq 11 and with the proper choice of the normalization constant, one can obtain the free energy potential function $\tilde{F}^{(N)}(\mathbf{x})$ to describe the thermodynamics of the helical peptide embedded in solvent

$$\tilde{F}^{(N)}(\mathbf{x}) = \sum_{i=1}^N j_i^{(1)} x_i + \sum_{i=1}^{N-1} j_i^{(2)} x_i x_{i+1} + \sum_{i=1}^{N-2} j_i^{(3)} x_i x_{i+1} x_{i+2} - T \sum_{i=1}^N (x_i - 1) \Delta S_i^c \quad (15)$$

The end effects on hydrogen bonding⁹ can be accounted for in eq 15 by modifying the $j_i^{(3)}$ factors of the end units.

On a long enough time scale, the local movements of the unit i on the $F^{(N)}(\Omega)$ potential surface can be described as two-state transitions between the helix and the coil potential wells separated by a barrier.⁴⁵ Kinetics of the peptide chain can be described then as a sequence of single flip transitions in the \mathbf{x} space.¹² According to ref 14, the transition rate of the i th unit in the solvent and peptide environment is

$$w(x_i \rightarrow 1 - x_i) = k \frac{\exp(-\beta F^{(N)}(\Omega))}{\exp(-\beta F^{(N)}(\mathbf{x}))} \quad \Omega_i = \Omega_i^+ \quad (16)$$

where Ω_i^+ is the transition-state conformation of the unit i . The coefficient k depends on the shape of the multidimensional free

energy potential $F^{(N)}(\Omega)$ and friction coefficients with the surrounding (see review in ref 46). The master equation for single flip transitions reads¹²

$$\frac{dP(\{x_1, \dots, x_i, \dots\}, t)}{dt} = - \sum_i w(x_i \rightarrow 1 - x_i) P(\{x_1, \dots, x_i, \dots\}, t) + \sum_i w(1 - x_i \rightarrow x_i) P(\{x_1, \dots, 1 - x_i, \dots\}, t) \quad (17)$$

where $P(\{x_1, \dots, x_i, \dots\}, t)$ is the probability of occupying the state $\mathbf{x} = \{x_1, \dots, x_i, \dots\}$ at the moment t .

3. Free Energy Factors for Alanine-Based Peptides Embedded in Water

To estimate the free energy factors $j_A^{(m)}$, $m = 1, \dots, 3$ (the subscript A denotes an alanine), in the potential function in eq 15 one has to find the helix Ω_A^1 and coil Ω_A^0 conformations in eq 10. The helix minimum is close to the canonical α -helix torsional angles^{18,22,36} $\Omega_A^1 \approx (\varphi = -57^\circ, \psi = -47^\circ)^1$. Recent experiments showed^{47–49} that the coil state of short AP in water is mainly populated by polyproline II conformations $\Omega_A^0 \approx (\varphi = -75^\circ, \psi = 145^\circ)^1$. These Ω_A^0, Ω_A^1 conformations were used as the approximations to the exact minima of the $F^{(N)}(\Omega)$ potential.

Alanine $j^{(m)}$ Free Energy Factors. According to experimental results,¹⁹ we assume that the energy parts $\Delta E_A^{(m)}$ of the $j^{(m)}$ factors do not depend on temperature

$$j_A^{(m)} = \Delta E_A^{(m)} - T\Delta S_A^{(m)s} \quad m \in (1, 2, 3) \quad (18)$$

QM calculations⁵⁰ of alanine dipeptides with different solvation models showed qualitatively similar results for $\Delta E_A^{(1)}$, but quantitatively the differences between the results of these models are ~ 1 kcal/mol. Using eq 15 and the results of QM calculations²² for N -alanine with the Poisson–Boltzmann continuum model of solvation ($N = 1, \dots, 7$), we found that $\Delta E_A^{(1)} \approx 1.5$ kcal/mol and $\Delta E_A^{(2)} \approx -1.2$ kcal/mol. Using eqs 15 and 18, one can find the energy of helix elongation

$$\Delta E^h = \Delta E_A^{(1)} + \Delta E_A^{(2)} + \Delta E_A^{(3)} \quad (19)$$

Assuming that the change of the volume is small in helix folding, we used the experimental value of approximately -0.9 kcal/mol for the enthalpy of helix elongation of AP–water systems¹⁹ as ΔE^h in eq 19 and found $\Delta E_A^{(3)} \approx -1.2$ kcal/mol. To account for the end effects on hydrogen bonding,⁹ we assumed

$$\Delta E_{N,C}^h = \Delta E^h + \Delta \Delta E_{N,C}^{(3)} \quad (20)$$

where $\Delta \Delta E_{N,C}^{(3)}$ are the corrections at the N- and C-termini, respectively. From the average QM results²² for N -alanine peptide ($N = 4, 5$), we estimated $\Delta E_N^h \approx -0.95$ kcal/mol and $\Delta E_C^h \approx -0.25$ kcal/mol. Using these values, one can find from eq 20 that $\Delta \Delta E_N^{(3)} \approx -0.05$ kcal/mol and $\Delta \Delta E_C^{(3)} \approx 0.7$ kcal/mol for alanine.

The small hydrophobic effect $\Delta S_A^{(1)s} \approx 0.7$ cal/(molK) due to the change of the accessible surface area (ASA) was estimated in ref 25. For the adjacent units the ASA change was assumed to be the sum of the ASAs of the separate units. Using this assumption, one can find from eqs 15 and 18 that $\Delta S_A^{(2)s} = 0$. The $\Delta S_A^{(3)s}$ entropy contribution is defined by the change of the number of hydrogen bonds in water upon backbone hydrogen bonding. We assumed that in helix folding two hydrogen bonds between water and polar groups of the backbone are

TABLE 1: Free Energy Factors of Alanine (A) and Arginine (R) Used for MC Simulations^a

	$j^{(1)}$		$j^{(2)}$		$j^{(3)}$				
	$\Delta E^{(1)}$	$T\Delta S^{(1)s}$	$\Delta E^{(2)}$	$T\Delta S^{(2)s}$	$\Delta E^{(3)}$	$T\Delta S^{(3)s}$	$T\Delta S^c$	$\Delta \Delta E_N^{(3)}$	$\Delta \Delta E_C^{(3)}$
A	1.5	0.2	-1.2	0	-1.2	1.2	-1.8	-0.05	0.7
R	1.5	0.2	-1.2	0	-1.4	1.2	-3.6		

^a All values are given in kcal/mol for temperature $T = 273.1$ K.

replaced with one hydrogen bond between polar groups of backbone and one hydrogen bond between water molecules that were expelled from the backbone. An entropy cost of ~ 6.7 cal/(mol K) of a water molecule binding to a polar group was estimated in ref 33, and an entropy cost of ~ 9 cal/(mol K) of water–water hydrogen bonding was estimated in ref 27. The resulting increase $\Delta S_A^{(3)s} \approx 4.4$ cal/(mol K) of the water entropy is favorable to helix folding.

Conformational Entropy of Alanine. It is reasonable to assume that the helix minimum of polypeptide free energy coincides with the region defined by the steric restrictions on backbone hydrogen bonding. To estimate the integrals in eq 14 we used the equilibrium bond length and the angles of the peptide backbone due to statistical analysis of X-ray data⁵¹ and the experimental criteria for protein hydrogen bonding⁵² (Appendix A). We used a hard-sphere⁴³ potential to calculate the number of excluded conformations of N -peptide in $2N$ -dimensional Ω space ($N = 1, \dots, 10$). In agreement with ref 43, we found the excluded conformations mainly in the coil region. Assuming a uniform decrease of the coil conformations for each dipeptide unit, we found $\gamma \approx 0.86$ for $N = 8, 9, 10$ (Appendix A). In agreement with results reported in refs 25 and 29, we found $\Delta S_A^c \approx -6.6$ cal/(mol K).

Free Energy Factors of Arginine. We estimated the change of the conformational entropy ΔS_R^c (subscript R denotes an arginine) as the sum of the side chain ΔS_R^{ch} and backbone ΔS_R^b entropy changes

$$\Delta S_R^c = \Delta S_R^b + \Delta S_R^{ch} \approx \Delta S_A^c + \Delta S_R^{ch} \quad (21)$$

Because ΔS_A^c describes the conformational entropy loss of the backbone, we assumed $\Delta S_R^b \approx \Delta S_A^c$ in eq 21. $\Delta S_R^{ch} \approx -6.7$ cal/(mol K) was estimated from the experimental data in ref 31. Using eq 21, one can find $\Delta S_R^c \approx -13.3$ cal/(mol K). The interactions of positively charged arginine side chain have to contribute to the energy of helix elongation.²⁰ We modeled this effect using $\Delta E_R^{(3)}$ as a fitting parameter in MC simulations while $\Delta E_R^{(1)}$, $\Delta E_R^{(2)}$, $\Delta S_R^{(1)s}$, and $\Delta S_R^{(2)s}$ were left with the same values as for alanine. The free energy factors of alanine and arginine are summarized in Table 1.

4. Results of MC Thermodynamics and Kinetics Simulations of F_s Peptide

F_s peptide has been extensively studied experimentally.^{53–55} From these experiments, it is known that F_s peptide has high helix propensity and fast ($\sim 10^{-6}$ s) exponential kinetics. Using the potential function (eq 15), MC thermodynamics simulations were performed with the Metropolis algorithm starting from a random configuration at high temperature. To reach the thermodynamic equilibrium and to average the quantities of interest, we used Markov's chains with lengths on the order 10^7 and 10^9 of MC steps per chain unit, respectively. The equilibrium temperature dependence of the helical fraction f_h (Figure 2 a) is in a good agreement with the experimental data⁵⁴ of UV resonance Raman spectroscopy (UVRS). MC simulations reproduced the broad temperature range character of the thermal

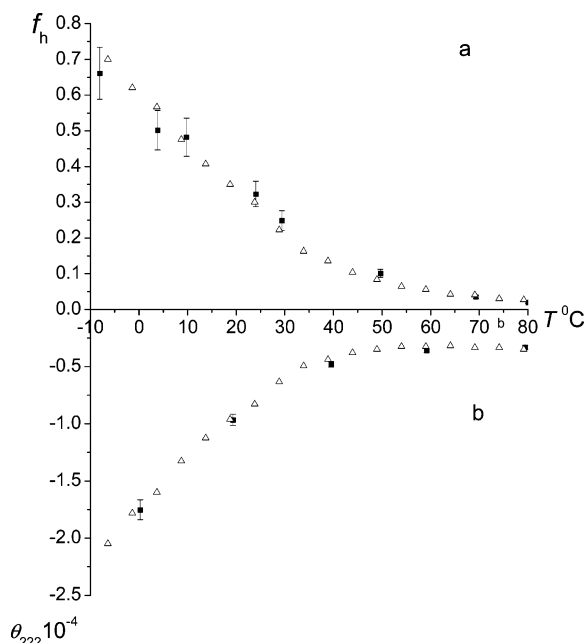


Figure 2. Equilibrium temperature dependences of (a) the helical fraction f_h and (b) the 222 nm molar ellipticity θ_{222} in deg cm² dmol⁻¹: Δ , MC simulations; \blacksquare , experimental results.⁴⁷

folding–unfolding of AP systems^{3,53,54} with $T_{1/2} \cong 280$ K, where $T_{1/2}$ is the temperature at which the helical content is equal to 0.5. We fitted the measured temperature dependence of the molar ellipticity⁵⁴ (CD) at $\lambda = 222$ nm $\theta_\lambda(T)$ using the approach that was proposed in ref 3

$$\theta_\lambda(T) = (\theta_\lambda^h(T_0) + \Delta\theta_\lambda^h(T - T_0))f_h + (\theta_\lambda^c(T_0) + \Delta\theta_\lambda^c(T - T_0))(1 - f_h) \quad (22)$$

where $T_0 = 273.1$ K and $T \geq T_0$. The values of the coil ellipticity $\theta_\lambda^c(T_0) = 640^\circ$ cm² dmol⁻¹ and the coefficients of the temperature dependences of the coil and the helix ellipticity, $\Delta\theta_\lambda^c = -46^\circ$ cm² dmol⁻¹ K⁻¹ and $\Delta\theta_\lambda^h = 103^\circ$ cm² dmol⁻¹ K⁻¹ were taken from results in ref 3. An agreement with CD measurements (Figure 2 b) was obtained for the value of the helix ellipticity of $\theta_\lambda^h(T_0) = -29\,000^\circ$ cm² dmol⁻¹. This value of $\theta_\lambda^h(T_0)$ is close to the approximately $-26\,000^\circ$ cm² dmol⁻¹ value of the ellipticity of the fully folded F_s peptide in a solution of water and α -helix stabilizer.⁵⁴

Kinetic simulations to solve master eq 17 were performed using the dynamical MC algorithm proposed in ref 56. In simulations the rate for single flip transitions (Appendix B)

$$w(x_i \rightarrow 1 - x_i) = \tau^{-1} \exp(\beta \Delta F_i(x_i))$$

$$\Delta F_i(x_i) = x_i(j_i^{(1)} + j_{i-1}^{(2)}x_{i-1} + j_i^{(2)}x_{i+1} + j_{i-2}^{(3)}x_{i-2}x_{i-1} + j_{i-1}^{(3)}x_{i-1}x_{i+1} + j_i^{(3)}x_{i+1}x_{i+2}) - (x_i - 1)T\Delta S_i^c \quad (23)$$

was used with $\tau = \text{constant}$ for all conformations of the F_s peptide. The helix fraction of the unit i was averaged over $M = 10^3$ MC kinetic trajectories

$$\langle x_i(t) \rangle = \sum_{\mathbf{x}} x_i P(x_1, \dots, x_i, \dots, x_N, t) \approx \frac{1}{M} \sum_{m=1}^M x_{i,m}(t) \quad (24)$$

where $x_{i,m}(t)$ is the state of the unit i at the moment t on the trajectory m . The result of the simulation of the thermal unfolding of the F_s peptide after the 22 K temperature jump

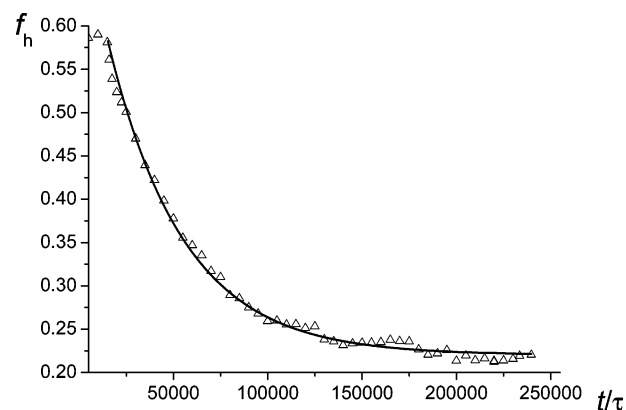


Figure 3. MC kinetics simulation of the thermal unfolding of the F_s peptide after a 22 K temperature jump from $T_0 = 277.1$ K to $T_1 = 299.1$ K: Δ , MC simulations. The black solid line is the monoexponential fit of the MC kinetics results.

from $T_0 = 277.1$ K to $T_1 = 299.1$ K (Figure 3) is in an agreement with experimentally observed monoexponential relaxation.⁵⁵ The fitting of MC simulations to the monoexponential behavior

$$f_h(t) = \frac{1}{N} \sum_{i=1}^N \langle x_i(t) \rangle \cong \bar{f}_h(T_1) + (\bar{f}_h(T_0) - \bar{f}_h(T_1)) \exp(-t/t_r) \quad (25)$$

(where \bar{f}_h is the equilibrium value helix fraction) gives the relaxation time $t_r \cong 40\,000\tau$. The experimental result for the relaxation time⁵⁵ $t_r = 180$ ns was used to estimate from eq 23 the time scales of the coil-to-helix $t_{0 \rightarrow 1} \approx 30$ ps and helix-to-coil $t_{1 \rightarrow 0} \approx 70$ ps transitions.

5. Discussion

Starting from the point that the existence of the helix and coil regions in the conformational space of the AP–water systems was confirmed by QM and MD/FF calculations, we further reexamined the expansion in eq 6 of the free energy potential in eq 2. We found that the neglected in LR theory nearest-neighbor interactions are of the same order as the interactions of hydrogen-bonded units. Omitting of the nearest-neighbor interactions was questioned previously in ref 8 and recently in ref 43. Using the partial charges of the polar groups⁵⁷ and a value of the dielectric constant of 80, we estimated the Coulomb interactions of i and $i + 2$ of the peptide bond to favor the helix conformation by approximately -1.3 kcal/mol, which is close to the value of $\Delta E_A^{(2)}$ (Table 1). The energy of the i and $i + 3$ peptide bond interactions can be viewed as the sum of the hydrogen-bonding, electrostatic, and van der Waals energies. A value of approximately -1 kcal/mol was estimated for the electrostatic part of the i and $i + 3$ interactions. These estimations omit all interactions other than electrostatic forces but give the physical reason for the equal $\Delta E_A^{(2)}$ and $\Delta E_A^{(3)}$ factors of alanine (Table 1) if we also take into account, as discussed in the Introduction, the small effect of hydrogen bonding on the energy of the AP–water system. The unfavorable contribution of the $\Delta E_A^{(1)}$ term reduces the $\Delta E_A^{(2)}$ and $\Delta E_A^{(3)}$ sum to the experimentally observed¹⁹ energy $\Delta E_A^{(h)} \cong -0.9$ kcal/mol.

Helix elongation is opposed by a backbone entropy loss of approximately -6.6 cal/(mol K), which is compensated for by the increase of the entropy of water molecules due to the change in ASA of ~ 0.7 cal/(mol K) and due to the additional degrees

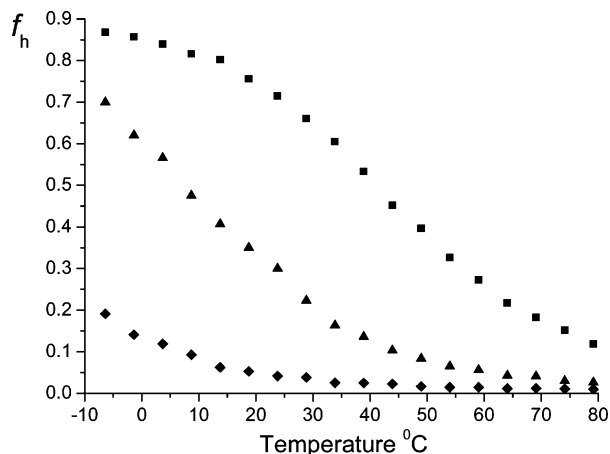


Figure 4. MC simulations of the equilibrium temperature dependences of the helical fraction f_h : ▲, with the parameters provided in Table 1; ■, with $\Delta E_{A,R}^{(3)} = -1$ kcal/mol, ◆, with $\Delta E_{A,R}^{(3)} = -0.8$ kcal/mol and all other parameters with the same values as in Table 1.

of freedom of the water molecules expelled from the backbone of ~ 4.4 cal/(mol K). The total change of entropy $\Delta S \approx -1.5$ cal/(mol K) is unfavorable for helix elongation. This result is close to but lower than the previous estimation⁴⁷ of $\Delta S \approx -2.2 \pm 0.4$ cal/(mol K), which was done using the enthalpy of helix elongation from the experiment¹⁹ and the phenomenological result of the LR theory with an accounting of the N- and C-terminal effects.⁵⁸ In line with the previous results for non-alanine amino acids,⁵⁹ we found the N-terminus to favor the helix folding of alanine (Table 1). In MC simulations, we used $\Delta E_R^{(3)}$ of arginine as a fitting parameter. The result for $\Delta E_R^{(3)}$ shows the dependence of the arginine helix propensity on the energy of the side-chain interactions as was found experimentally in ref 20.

The model free energy potential in eq 15 was applied successfully to thermodynamics and kinetics of the F_s peptide. In kinetics simulations, we used the constant value of τ in eq 23; thus implicitly τ was averaged over the conformational space. MD simulations could be used to calculate the transition rates in eq 23 microscopically to solve master eq 17. The two-state description of kinetics works if there are no kinetic traps in the coil state,⁴⁵ which were supposed to be the reason for the slow ($\sim 10^{-3}$ s) multistep folding observed for poly-L-glutamic acid peptides.⁶⁰ We want to emphasize that the quantitative description of helix folding is a challenge for researchers because even a small change in the value of the helix propensity substantially changes the helix fraction of the AP–water solution (Figure 4).

6. Conclusions

α -Helix folding in AP–water systems is known to be driven by the enthalpy of molecular interactions and opposed by the entropy loss of the systems. In the frame of the proposed model, the underlying contributions to both the enthalpy and the entropy of helix elongation of polyalanine peptides in water were estimated microscopically to model the mechanism of α -helix folding. At the current level of QM calculations, the formalism of the LR theory can be generalized to describe from a uniform point of view α -helix folding in heteropeptide systems.

Appendix A: Details of the Estimation of Alanine Conformational Entropy

We used the equilibrium bond lengths and angles of the backbone⁵¹ and the α -helix torsional angles to calculate the coordinates of the atoms of the i , $i + 1$, and $i + 2$ peptide bond.

The coordinates of the atoms of the $i + 3$ peptide bond were calculated for each point of Ω space with a 1° step over the φ, ψ directions. Each conformation was added to the number of helix conformations L^h if the steric structure of the peptide satisfied the experimental criteria for protein hydrogen bonding.⁵² Otherwise it was added to the number of coil conformations L^c , provided that the point was not prohibited by the hard-sphere potential.⁴³ After enumerating all possible conformations of Ω space, we obtained L^h and L^c , which are proportional to the areas of the helix and coil regions, respectively. To estimate the excluded volume of the conformational space of the peptide which consists of N alanine dipeptide units the number M_N of all allowed by hard-sphere potential conformations were calculated in $2N$ -dimensional Ω space with the 30° step over the φ, ψ directions for $N = 1, \dots, 10$. With this accuracy, the excluded conformations are due to the decrease of the number of available coil conformations of the alanine dipeptide

$$M_N = (\gamma M^c + M^h)^N \quad \gamma < 1 \quad (\text{A1})$$

where M^c and M^h are the numbers of the coil and helix conformations of the alanine dipeptide calculated similar to L^c and L^h but with a 30° step. From eq A1, we found $\gamma \approx 0.86$ for $N = 8, 9, 10$. Using eq 14, one can estimate the conformational entropy change per mole of the dipeptide units of the polyalanine peptide in the helix-to-coil transition

$$\Delta S_A^c = R \ln \frac{\int \delta(x'(\Omega_i) - x_i) \gamma(x_i) d\Omega_i}{\int \delta(x'(\Omega_i)) \gamma(0) d\Omega_i} \approx R \ln \gamma \frac{L^h}{L^c} \approx -6.6 \text{ cal/(mol K)} \quad (\text{A2})$$

where R is the universal gas constant and $L^c/L^h \approx 35.3$ due to the above-described enumerating algorithm.

Appendix B: The Rate of Single Flip Transition

One can rewrite eq 9 as

$$F^{(N)}(\Omega) \approx \sum_{k=1, k \neq i}^N j_k^{(1)} x_k + \sum_{k=1, k \neq i-1, i}^{N-1} j_k^{(2)} x_k x_{k+1} + \sum_{k=1, k \neq i-2, i-1, i}^{N-2} j_k^{(3)} x_k x_{k+1} x_{k+2} + \Delta F_i(\Omega) + \tilde{j}^{(N)}(\Omega), \Omega_i = \Omega_i^+ \quad (\text{B1})$$

and denote

$$\Delta F_i^+ = \Delta F_i(\Omega_1, \dots, \Omega_i^+, \dots, \Omega_N)$$

$$\Delta F_i(x_i) = x_i(j_i^{(1)} + j_{i-1}^{(2)} x_{i-1} + j_i^{(2)} x_{i+1} + j_{i-2}^{(3)} x_{i-2} x_{i-1} + j_{i-1}^{(3)} x_{i-1} x_{i+1} + j_i^{(3)} x_{i+1} x_{i+2}) - (x_i - 1) T \Delta S_i^c \quad (\text{B2})$$

where in both eqs B1 and B2 Ω_i^+ is the transition-state conformation of the unit i between its helix and coil regions. Using eqs 9, B1, and B2 and assuming that $\tilde{j}^{(N)}(\Omega)$ is zero if $\Omega_i = \Omega_i^+$, one can obtain

$$w(x_i \rightarrow 1 - x_i) \approx k \exp[-\beta(\Delta F_i^+ - \Delta F_i(x_i))] = \tau^{-1} \exp[\beta \Delta F_i(x_i)] \quad (\text{B3})$$

where $\tau = k^{-1} \exp(-\beta \Delta F_i^+)$ depends on the activation barrier ΔF_i^+ and k .

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