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Prediction of α-Helix Folding of Isolated C-Peptide of Ribonuclease A by Monte Carlo Simulated Annealing

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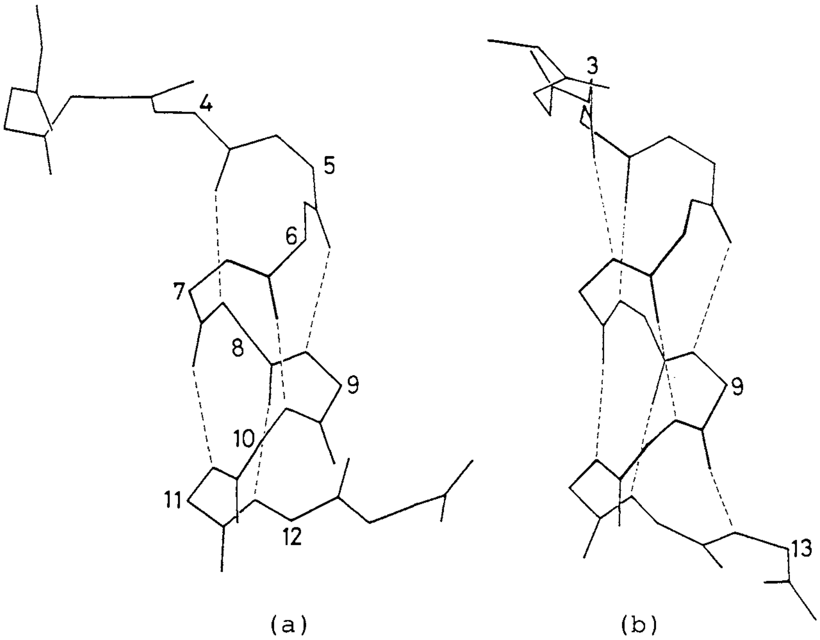
Conformation of the C-peptide fragment of RNase A is calculated by Monte Carlo simulated annealing. Starting from completely random initial conformations and minimizing the total potential energy without any bias, we obtained partial α-helix structure with a high probability ($\approx 40\%$). The energetically most favourable structure exhibits a 2.5-turn α-helix. The results of simulation are consistent with the experimental implication for the role of charged residues for α-helix stability in this peptide.

The difficulty in predicting the folded structure of a protein or its fragment lies in the fact that there exist a huge number of local minima in the energy function. The calculation of conformation so far made employs information of the empirical structure, imposing a certain bias towards a favoured final state. There are, however, a few methods known to statistical physicists that might alleviate the difficulty. The Monte Carlo simulated annealing method[1)] was first applied to the conformational prediction without the use of empirical data in Ref. 2. In this communication we report on our application of the method to the C-peptide fragment of RNase A (residues 1–13 of ribonuclease A). Starting from completely random initial conformations, we minimized the total potential energy given by the sum of generic interatomic energies whose parameters are determined separately for each amino acid. The simulation was carried out in a completely unrestricted way without imposing any weight towards helical structure. We have obtained partial α-helix structure with a high probability ($\approx 40\%$) close to the C-terminus in agreement with the results of X-ray crystallography[3)] and solution studies.[4–8)] The simulation with varied charges of the residues sheds light on the α-helix stability problem; for instance, our results support the experimental implication of the importance of intramolecular ionic interactions for α-helix stability in this peptide.[7,8]

We considered the peptides with both neutral and charged Glu-9. Since neutral Glu-9 gives more conspicuous α-helix structure, we mainly report on the results for this case. In the present simulation we improved our previous annealing algorithm[2] and vectorized the program to reduce large computation time. The semi-empirical potential energy function that we used is given by the sum of electrostatic, van der Waals (with a reduced 1-4 interaction strength), hydrogen-bond, and torsion energy with their parameters given in Ref. 9. The effect of the solvent was neglected, and the dielectric constant was set equal to 2 according to Ref. 9. We minimized the total potential energy with respect to 13 set of dihedral angles (ϕ_i, ψ_i) $(i=1, \cdots, 13)$ and 38 torsion angles in amino acid residues with the peptide-bond dihedral angles ω set equal to π. We made a sequential update of the above 64 angles allowing their range from $-\pi$ to π at each Monte Carlo step. In order to surmount the multiple-minima problem, annealing is incorporated as follows. Starting simulations at a sufficiently high temperature, we lower the temperature at each Monte Carlo step by a small amount until it is low enough. If the annealing is successful, we should reach close to the global minimum at the end of the run.[1]

In the present simulation we lowered the temperature exponentially. We first made 250 short runs with 1000 Monte Carlo steps to obtain an idea for the optimal initial temperature. The initial temperatures for these runs were 5000 K, 3000 K, 1000 K, 500 K, and 300 K with the final temperature always fixed to 300 K. The final temperature was set to this value, since we did not detect large changes in conformation from Monte Carlo step to step close to 300 K. The runs starting at 1000 K gave conformations with the minimum mean potential energy among the above five cases. Thus, setting the initial temperature to 1000 K and the final temperature to 250 K, we made 20 long runs with 10000 Monte Carlo steps. The computation (CPU) time spent for each run with 10000 Monte Carlo steps was roughly 10 minutes on a HITAC S-820/80 computer. The initial conformations as well as sampling at each Monte Carlo step were all taken to be random and uncorrelated, and the Monte Carlo procedure was fully unrestricted. Out of 20 final conformations obtained, eight exhibited partial α-helix structure with 1–2.5 turns. This may be compared with the tertiary structure deduced from the X-ray diffraction data reported for RNase S,[3] which indicates three turns of α-helix in Thr-3 to Met-13. For the isolated C-peptide in water near 0 °C circular dichroism spectra indicate that the partial α-helix remains with the fraction of 30%.[4–6] Our lowest-energy conformation among the 20 final conformations indeed exhibits a 2.5-turn α-helix; Ala-4 to His-12 forms an α-helix with hydrogen bonds of $\approx$ 1 kcal/mol between $(CO)_i$ and $(NH)_{i+4}$ $(i=4, \cdots, 8)$. This structure is presented in Fig. 1 for the backbone together with the empirical conformation of the residues 1–13 of native RNase A,[3] and the dihedral angles of the two conformations are compared in Table 1. As is clear from Fig. 1, the overall similarity between the two is apparent. The number of our Monte Carlo runs, while it is perhaps not large enough to explore the detailed structure of the C-peptide, seems sufficient to show that the α-helix appears in the vicinity of the global minimum.



(a) (b)

Fig. 1. Our lowest-energy conformation (*a*) and the conformation constructed from from the X-ray data (*b*) (backbone only). The broken lines represent hydrogen bonds between $(CO)_i$ and $(NH)_{i+4}$. The residue numbers represent the locations of C_i^{α} $(i = 1, \cdots, 13)$.

Table 1. Dihedral angles of our lowest-energy conformation and those deduced from the X-ray data

	Calculation		X-Ray data	
Residue	ϕ	ψ	ϕ	ψ
Lys-1$^+$	−2	−21		−5
Glu-2$^-$	−89	−19	−99	160
Thr-3	−107	22	−127	172
Ala-4	−67	134	−43	−64
Ala-5	−63	−28	−62	−43
Ala-6	−67	−40	−60	−56
Lys-7$^+$	−64	−46	−39	−64
Phe-8	−58	−42	−23	−72
Glu-9	−72	−35	−63	−68
Arg-10$^+$	−61	−43	−40	−48
Gln-11	−79	−48	−70	−47
His-12$^+$	−135	101	−95	1
Met-13	−60	146	−112	155

We also made simulations with charged Glu-9. Out of 20 runs with 10000 Monte Carlo steps, eight conformations exhibited partial α-helix structure. The number of turns, however, tends to be reduced (1–1.5 turns). This is in accord with the observation[7,8] that the charged Glu-9 is not necessary for helix formation and that the substitution Glu-9^- → Leu-9 increases helix stability. Moreover, when both Glu-2 and His-12 were neutralized, no helix structure was obtained. This fact supports the experimental implication of the importance of these charged residues for α-helix stability in this peptide.[7,8] The coincidence of calculation and experimental facts suggests that the effects of solvent molecules are indeed small. In order to study further the effects of electrostatic interactions, we performed simulations with a large dielectric constant and obtained similar α-helix structure. The detailed discussion will be given elsewhere.[10]

Finally, let us remark that no obvious helical structure was found when we made conventional Monte Carlo simulations at a fixed temperature of 300 K. This demonstrates the importance of annealing procedure[2] in finding the conformation with the global-minimum energy.

The present version of our FORTRAN program, KONF90, is available from one of the authors (Y.O. at Department of Physics, Nara Women's University).

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