Tertiary structure prediction of C-peptide of ribonuclease A by multicanonical algorithm

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ABSTRACT

We have performed multicanonical Monte Carlo simulations of C-peptide of ribonuclease A. It is known by CD and NMR experiments that this peptide has high α-helix content in aqueous solution and that the side-chain charges of residues Glu-2− and His-12+ play an important role in the stability of the α-helix. In order to confirm these experimental implications, we have used two analogues of the peptide with charged and neutral side chains of Glu-2 and His-12. Two dielectric functions, distance-dependent and constant, are considered to study the effects of solvent contributions. All the simulations were started from random initial conformations. Various thermodynamic quantities such as average helicity as a function of residue number and average distance between two side chains as a function of temperature are calculated. The results are found to be in accord with the implications of CD and NMR experiments. The lowest-energy conformation obtained has an α-helix from Ala-4 to Gln-11 in complete agreement with the corresponding structure deduced from an X-ray crystallography experiment of ribonuclease A. It is shown that the salt bridge between the side chains of Glu-2− and Arg-10+, which is known to exist from both NMR and X-ray experiments, is formed only when the side chains are properly charged. Its formation is greatly enhanced when the distance-dependent dielectric function is used.

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The C-peptide, residues 1–13 of ribonuclease A, is known by CD and NMR experiments to have significant $\alpha$-helix formation in aqueous solution at temperature near 0 °C [1, 2]. In this article, we employ a multicanonical Monte Carlo simulation [3] to study the $\alpha$-helix stability of C-peptide due to the side-chain electrostatic interactions. The results are found to be in accord with various implications of the above experiments. The lowest-energy conformation obtained by the simulation has an $\alpha$-helix from Ala-4 to Gln-11 in complete agreement with the corresponding structure deduced from an X-ray crystallographic experiment of the whole ribonuclease A [4]. It is shown that the characteristic salt bridge between Glu-2$^-$ and Arg-10$^+$, which is known to exist both in the NMR experiment [2] and in the X-ray experiment [4], is formed with significant probability only when the side chains are properly charged and some solvation effects are included.

The CD experiment of C-peptide showed that the side-chain charges of residues Glu-2$^-$ and His-12$^+$ play an important role in the stability of the $\alpha$-helix, while the rest of the charges of other side chains do not [1]. A previous simulation work [5] by Monte Carlo simulated annealing [6] confirmed the $\alpha$-helix formation and the importance of the electrostatic interactions of the above two side chains for the stability of the helix. The simulation was performed in gas phase, however, and it failed in obtaining the characteristic salt bridge between Glu-2$^-$ and Arg-10$^+$.

In this work, we used two analogues of C-peptide in order to study the importance for $\alpha$-helix stability due to the electrostatic interactions of the side-chain charges of residues Glu-2 and His-12. The amino-acid sequences of these analogues are $K^+ETAAA+k^+FER^+QH^+M$ and $K^+ETAAA+k^+FER^+R^+QHM$. We refer to the former as Peptide I and the latter Peptide II hereafter. The main difference between the two peptides is the charges of residues Glu-2 and His-12 (both are charged for Peptide I and neutral for Peptide II, respectively).

The potential energy function that we used is given by the sum of the electrostatic term, 12-6 Lennard-Jones term, and hydrogen-bond term for all pairs of atoms in the peptide together with the torsion term for all torsion angles. The energy parameters were adopted from ECEPP/2 [7]. A distance-dependent dielectric function [8] was used to mimic the presence of water. A constant dielectric function ($\epsilon = 2$) was also used for a comparison.
with gas-phase simulations. The computer code KONF90 [3] was used.

The Monte Carlo method that we used is multicanonical algorithm [3], which belongs to a class of *generalized-ensemble algorithms* (for a discussion and comparison of these algorithms, see, for instance, Ref. [3]). This method was introduced to the protein folding problem a few years ago [10], and the effectiveness of the method has been established for oligopeptide systems [11, 12]. The method allows one to sample a wide range of configuration space and overcome the multiple-minima problem that is responsible for the very long equilibration time required by conventional methods. From a single simulation run, it thus enables one to obtain not only the lowest-energy conformation but also any thermodynamic quantity over a wide range of temperatures [3, 11]. In the present work, we performed four multicanonical Monte Carlo simulations of 1,000,000 Monte Carlo sweeps each, where one Monte Carlo sweep updates all the torsion angles in the peptide once. The four runs are: one with distance-dependent dielectric function for Peptide I, one with constant dielectric function for Peptide I, and two corresponding runs for Peptide II. The simulations were started from completely random initial conformations.

We first examine how much α-helix formation we obtain by the simulations. We found that on the average 65.2 (1.0) % of the residues are in α-helix state at temperature $T = 273$ K for Peptide I with distance-dependent dielectric function, while the value was 52.3 (3.5) % for Peptide II with the same dielectric function (the numbers in parentheses are errors). Here, a residue is defined to be in α-helix state if the backbone dihedral angles ($\phi, \psi$) fall in the range ($-70 \pm 30^\circ, -37 \pm 30^\circ$). The average length of α-helix at this temperature are, likewise, 7.7 (2.2) residues long and 4.8 (0.5) residues long for Peptide I and Peptide II, respectively. Furthermore, at this temperature the average energy differences between helical conformations and non-helical conformations, $\Delta E (\equiv < E_H > - < E_C >)$, are $-20.5$ (3.5) and $-3.1$ (4.7) for Peptide I and Peptide II, respectively. Here, $< E_H >$ and $< E_C >$ stand for the average total potential energies of helical conformations and non-helical conformations, respectively (and a helical conformation is defined to be a conformation that has at least 3 successive residues in the α-helix state). The large difference in $\Delta E$ implies that a helical conformation is energetically favored. All these results support the experimental fact that the side-chain charges of the residues Glu-2
and His-12 enhance the α-helix stability of C-peptide [1].

In Figure 1 we compare the average % helicity of the two peptides at $T = 273$ K as a function of residue number obtained by simulations with the distance-dependent dielectric function. The overall helicity is larger in Peptide I than in Peptide II as just discussed above. The helicity is very high from residue 4 to residue 11 and it is very low from residue 1 to residue 3 (and residues 12 and 13) for Peptide I. This is in accord with the implications of the NMR experiment of C-peptide, where they found lowered population of helices for residues 1–3 and high helix content for residues 5–12 [2]. The results for Peptide II, on the other hand, is inconsistent with the NMR data in that they predict high helicity for residue 2 and very low helicity for residue 7. These findings again support the fact that the charges of the residues Glu-2 and His-12 are important for the α-helix stability of C-peptide.

The lowest-energy conformations of the two peptides obtained by the simulations with the distance-dependent dielectric function are now compared. In Table I we give the backbone dihedral angles of these conformations together with those of a structure deduced by the X-ray experiment [4]. The conformation of Peptide I has an α-helix in residues 4–11 in complete agreement with the X-ray data, while that of Peptide II has an extended α-helix only for residues 8–12. The root-mean-square (r.m.s.) deviations of these conformations from the X-ray structure are also presented in the Table. One finds that the backbone structure for Peptide I is very similar to that of the X-ray data (r.m.s. distance of 1.4 Å).

The lowest-energy conformation of Peptide I is shown in Figure 2A. From the Figure we see that the characteristic salt bridge between Glu $2^-$ and Arg $10^+$, which exists both in the NMR data [2] and in the X-ray data [4], is indeed formed. In Figure 2B this structure and the X-ray structure are displayed together in a superposition. One can see that the two tertiary structures are quite similar to each other (r.m.s. distance is 2.7 Å from Table I).

The formation of the salt bridge can be studied by calculating the average distance between the side chains of Glu-2 and Arg-10 as a function of temperature. Here, the distance between these side chains is defined to be the smallest of the distance between
O of Glu-2 and H of Arg-10. The results for all four runs are given in Figure 3. From the Figure one finds that the results for Peptide I with distance-dependent dielectric function give the shortest average distance between the two side chains at low temperatures. This implies that the salt bridge between Glu-2 and Arg-10 is favored most when the side chains of residues Glu-2 and His-12 are charged (Peptide I rather than Peptide II) and some solvation effects are included (distance-dependent dielectric function rather than constant one).

In this article, we have presented the results of multicanonical Monte Carlo simulations applied to the tertiary-structure prediction of C-peptide of ribonuclease A. The results were in good agreement with various implications of CD, NMR, and X-ray experiments. It should be emphasized that the simulations were performed from completely random initial conformations and that no structural information from experiments was used as input. Furthermore, it is a great advantage of multicanonical algorithm over other methods that one needs only a single simulation run to obtain any thermodynamic quantity for a wide range of temperatures.

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Table I. Backbone dihedral angles (in degrees)\(^a\) of the lowest-energy conformations of Peptides I and II for distance-dependent dielectric function obtained from the multicanonical simulations and those deduced from the X-ray data \(^b\), together with the r.m.s. deviations (in Å) from the X-ray structure.\(^b\)

| Residue | X-ray | Peptide I | Peptide II |
|---------|-------|-----------|------------|
|         | φ     | ψ         | φ          | ψ          | φ          | ψ          |
| Lys-1   |       | 175       | −7         | −51        | −2         | −41        |
| Glu-2   | −58   | 136       | −83        | 79         | −60*       | −34*       |
| Thr-3   | −68   | 159       | −102       | 156        | −79        | 69         |
| Ala-4   | −59*  | −45*      | −57*       | −32*       | −69        | 125        |
| Ala-5   | −64*  | −48*      | −70*       | −49*       | −159       | 166        |
| Ala-6   | −64*  | −34*      | −59*       | −37*       | −57*       | −41*       |
| Lys-7   | −63*  | −42*      | −64*       | −52*       | −62        | 116        |
| Phe-8   | −61*  | −42*      | −61*       | −36*       | −55*       | −38*       |
| Glu-9   | −58*  | −46*      | −63*       | −48*       | −55*       | −47*       |
| Arg-10  | −64*  | −37*      | −62*       | −36*       | −75*       | −33*       |
| Gln-11  | −71*  | −28*      | −72*       | −37*       | −57*       | −40*       |
| His-12  | −120  | −12       | −172       | 120        | −86*       | −41*       |
| Met-13  | −104  | 130       | −60        | 133        | −106       | 98         |

R.m.s. deviation from X-ray structure

|                     | Backbone | Peptide I | Peptide II |
|---------------------|----------|-----------|------------|
| Backbone            | 0.0      | 1.4       | 5.2        |
| All Atoms           | 0.0      | 2.7       | 6.1        |

\(^a\) The asterisks indicate that the corresponding residues are in the \(\alpha\)-helix state, where a residue is defined to be in \(\alpha\)-helix state if the dihedral angles \((\phi, \psi)\) fall in the range \((-70 \pm 30^\circ, -37 \pm 30^\circ)\).

\(^b\) The X-ray structure was taken from the Brookhaven Protein Data Bank file 8RAT \(^4\). The r.m.s. distance was calculated with respect to non-hydrogen atoms only.
Figure Legends

- Figure 1: Average % helicity of C-peptide analogues, Peptide I (PI) and Peptide II (PII), at $T = 273$ K as a function of residue number. The results are for the distance-dependent dielectric function. Here, a residue is defined to be in $\alpha$-helix state if the backbone dihedral angles ($\phi, \psi$) fall in the range ($-70 \pm 30^\circ, -37 \pm 30^\circ$). Each result was obtained from a multicanonical simulation of 1,000,000 Monte Carlo sweeps.

- Figure 2: (A) The lowest-energy conformation of C-peptide of ribonuclease A (Peptide I) obtained by a multicanonical simulation of 1,000,000 Monte Carlo sweeps with distance-dependent dielectric function. The side-chain atoms are suppressed except for those of Glu-2$^-\text{ and Arg-10}^+$ that form a salt bridge. These side chains are labeled in the figure. The N terminus and the C terminus are also labeled by N and C, respectively. The figure was created with Molscript [13]. (B) The lowest-energy conformation of Figure 2A (black sticks) and the corresponding X-ray structure (gray sticks) [4] superposed. All the atoms in the backbone and side chains are shown here, but the hydrogen atoms are suppressed. The N terminus and the C terminus are labeled by N and C, respectively. The figure was created with RasMol [14].

- Figure 3: Average distance $\langle d(2-10) \rangle$ (in Å) between the side chains of Glu-2 and Arg-10 as a function of temperature (in K). Here, the distance $d(2-10)$ is defined to be the smallest of the distance between $O^\epsilon$ of Glu-2 and $H^g$ of Arg-10. PI and PII correspond to Peptide I and Peptide II, respectively. $\epsilon = \text{dis}$ and $\epsilon = 2$ stand for distance-dependent dielectric function and constant dielectric function, respectively. Each result was obtained from a multicanonical simulation of 1,000,000 Monte Carlo sweeps.
DISTANCE-DEPENDENT EPSI: T=273 K

% Helicity vs Residue Number
GLU2 - ARG10 DISTANCE

PI: epsi=dis
PI: epsi=2
PII: epsi=dis
PII: epsi=2