Protein Folding Simulations by Simulated Annealing and Generalized-Ensemble Algorithms

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Monte Carlo simulated annealing and generalized-ensemble algorithms for protein folding problem are described in detail. Two oligopeptides, Met-enkephalin and C-peptide of ribonuclease A were studied. Only the amino-acid sequence information was used as input and initial conformations were randomly generated. The lowest-energy conformation obtained for C-peptide has an α-helix structure in remarkable agreement with experimental results.

§1. Introduction

In protein and other complex systems, simulations at low temperatures tend to get trapped in a few of huge number of local-minimum-energy states. One way to overcome this multiple-minima problem is by Monte Carlo simulated annealing,[1] which is perhaps the most widely used optimization method. Another effective way is to perform a simulation based on non-Boltzmann probability weight factors so that a random walk in energy space may be realized. Random walks allow the simulation to escape from any energy barrier and sample much wider phase space than by conventional methods. Monitoring the energy in a single simulation run, one can obtain not only the global-minimum-energy state but also any thermodynamic quantities as a function of temperature for a wide temperature range by the reweighting techniques. Well-known examples of such methods are multicanonical algorithm[2] and simulated tempering.[3),4] These methods that perform random walks in energy space due to non-Boltzmann weight factors are now given a generic name: generalized-ensemble algorithm.[5] (For reviews of generalized-ensemble approach in the protein folding problem, see Refs. 6), 7).)

In this article we discuss the uses of Monte Carlo simulated annealing and generalized-ensemble algorithms in the protein folding problem.

§2. Energy functions of protein systems

The conformational energy function E_P (in kcal/mol) for the protein molecule that we used is one of the standard ones. Namely, it is given by the sum of the electrostatic term E_C, 12-6 Lennard-Jones term $E_{\rm LJ}$, and hydrogen-bond term $E_{\rm HB}$

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for all pairs of atoms in the molecule together with the torsion term $E_{\rm tor}$ for all torsion angles.

One of the simplest ways to represent solvent effects is by the sigmoidal, distance-dependent dielectric function.[8] The explicit form of the function we used is given in Ref. 9). The distance-dependent dielectric function is simple and also computationally only slightly more demanding than the gas-phase case.

§3. Simulation methods

Once the appropriate energy function of the protein system is given, we have to employ a simulation method that does not get trapped in states of energy local minima. We have been advocating the uses of Monte Carlo simulated annealing[1] and generalized-ensemble algorithms (for reviews, see Refs. 6), 7)).

3.1. *Simulated annealing*

In the regular canonical ensemble with a given inverse temperature $\beta \equiv 1/k_B T$, the probability distribution of energy is given by

$$P_B(T,E) \;\propto\; n(E)W_B(E) \;=\; n(E)\exp\left(-\beta E\right) \;, \qquad (3\cdot1)$$

where $n(E)$ is the density of states with energy E. Since the density of states $n(E)$ is a rapidly increasing function of E and the Boltzmann factor $W_B(E)$ decreases exponentially with E, the probability distribution $P_B(T,E)$ has a bell-like shape in general. However, it is very difficult to obtain canonical distributions at low temperatures with conventional simulation methods. This is because the thermal fluctuations at low temperatures are small and the simulation will certainly get trapped in states of energy local minima.

Simulated annealing[1] is based on the process of crystal making. Namely, by starting a simulation at a sufficiently high temperature (much above the melting temperature), one lowers the temperature gradually during the simulation until it reaches the global-minimum-energy state (crystal). If the rate of temperature decrease is sufficiently slow so that thermal equilibrium may be maintained throughout the simulation, only the state with the global energy minimum is obtained (when the final temperature is 0 K). However, if the temperature decrease is rapid (quenching), the simulation will get trapped in a state of energy local minimum.

Our group has been testing the effectiveness of the method mainly in oligopeptide systems (for a review, see Ref. 6)).

3.2. *Generalized-ensemble algorithms*

While a regular Monte Carlo method generates states according to the canonical distribution, generalized-ensemble algorithms generate states so that a one-dimensional random walk in a pre-chosen physical quantity (for instance, the energy) is realized. Hence, any energy barrier can be overcome, and one can avoid getting trapped in states of energy local minima.

Multicanonical algorithm,[2] which is also referred to as entropic sampling[10] and adaptive umbrella sampling,[11] is one of the most well-known such methods. The

weight factor for multicanonical ensemble is given by

$$W_{\rm mu}(E) \propto \frac{1}{n(E)} = e^{-S(E)} \ , \tag{3·2}$$

where

$$S(E) = \log n(E) \tag{3·3}$$

is the microcanonical entropy. The generated configurations are in equilibrium with respect to the multicanonical distribution, not the Boltzmann distribution, and the simulation will lead to a uniform distribution of energy:

$$P_{\rm mu}(E) \propto n(E)\ W_{\rm mu}(E) = {\rm const} \ . \tag{3·4}$$

Since all energies appear with the equal probability, a free random walk in the energy space is enforced. Hence, the simulation can overcome any energy barrier and will not get trapped in one of the many local minima.

Since the multicanonical weight factor $W_{\rm mu}(E)$ is not *a priori* known, one has to determine it for each system by a few iterations of trial simulations. Once this weight factor is obtained, one performs a long production simulation run. The advantage of multicanonical algorithm lies in the fact that from this single production run, one can obtain not only the global-minimum-energy state but also the thermodynamic quantities for a wide range of temperatures. The latter is accomplished by the use of the single-histogram reweighting techniques.[12] Namely, the expectation value of a physical quantity $\mathcal{A}$ at temperature $T = 1/k_B\beta$ can be expressed in terms of the predetermined weight $W_{\rm mu}(E)$ and the obtained final distribution $P_{\rm mu}(E)$ as follows:

$$\langle \mathcal{A} \rangle_T \ = \ \frac{\int dE\ \mathcal{A}(E) P_{\rm mu}(E)\ W_{\rm mu}^{-1}(E)\ e^{-\beta E}}{\int dE'\ P_{\rm mu}(E')\ W_{\rm mu}^{-1}(E')\ e^{-\beta E'}} \ . \tag{3·5}$$

Closely related to the multicanonical algorithm is $1/k$ sampling.[13] Here the (microcanonical) entropy S is sampled uniformly:

$$P_{1/k}(S) = {\rm const} \ . \tag{3·6}$$

To realize such an ensemble, Hesselbo and Stinchcombe[13] proposed that configurations are assigned a weight

$$W_{1/k}(E) = \frac{1}{k(E)} \ , \qquad k(E) = \int_{-\infty}^{E} dE'\ n(E') \ . \tag{3·7}$$

Since the entropy $S(E)$ is a monotonically increasing function of energy, a random walk in entropy implies a random walk in energy space (with more weight towards low-energy region; compare Eqs. (3·2) and (3·7)).

Again, the weight $w_{1/k}(E)$ is not a priori known and its estimator has to be calculated. Thermodynamic quantities at any temperature can be calculated by Eq. (3·5), in which $P_{\rm mu}(E)$ and $W_{\rm mu}(E)$ are replaced by $P_{1/k}(E)$ and $W_{1/k}(E)$, respectively.

In *simulated tempering*,[3),4)] which is also referred to as expanded-ensemble method,[4)] temperature itself becomes a dynamical variable. Temperature and configuration are both updated with a weight:

$$W_{\rm ST}(T,E) = e^{-E/T-g(T)} \ , \tag{3·8}$$

where the function $g(T)$ is chosen so that the probability distribution of temperature is given by

$$P_{\rm ST}(T) = \int dE \ n(E) \ e^{-E/T-g(T)} = \text{const} \ . \tag{3·9}$$

Hence, in simulated tempering the temperature is sampled uniformly, while simulations in multicanonical and $1/k$ ensembles respectively sample energy and entropy uniformly. Physical quantities have to be sampled for each temperature point separately. Their expectation values at temperature T are then calculated in the usual way by

$$\langle \mathcal{O} \rangle_T \ = \ \frac{\int dx \ \mathcal{O}(x) \ e^{-E(x)/k_BT}}{\int dx \ e^{-E(x)/k_BT}} \ , \tag{3·10}$$

where x labels the conformations, and only those conformations that were obtained at temperature T are included in the integral.

As common in generalized-ensemble simulations, the weight $w_{\rm ST}(T,E)$ is not a priori known (since it requires knowledge of the parameters $g(T)$) and their estimator has to be calculated. They can be obtained by an iterative procedure again.

Despite their successful application to simulations of proteins and other complex systems, the above generalized-ensemble methods suffer from the problem that the determination of the weights can be non-trivial and tedious. We thus want to look for ensembles where the weight can be simply obtained.

One example is the ensemble[14)] that is based on Tsallis generalized statistical mechanics,[15)] where the problem is reduced to that of finding an estimator for a single quantity. We are interested in an ensemble where not only the low-energy region can be sampled efficiently but also the high-energy states can be visited with finite probability. In this way the simulation can overcome energy barriers and escape from local minima. The probability distribution of energy should resemble that of an ideal low-temperature Boltzmann distribution, but with a tail to higher energies. To obtain such an ensemble we proposed to update configurations according to the following probability weight:[14)]

$$w(E) = \left(1 + \frac{\beta(E-E_0)}{n_F}\right)^{-n_F} \ , \tag{3·11}$$

where E_0 is an estimator for the ground-state energy, n_F is the number of degrees of freedom of the system, and $\beta = 1/k_BT$ is the inverse temperature with a low temperature T. The weight reduces in the low-energy region to the canonical Boltzmann weight $\exp(-\beta E)$ for $\frac{\beta(E-E_0)}{n_F} \ll 1$. On the other hand, high-energy regions are no longer exponentially suppressed but only according to a power law, which enhances excursions to high-energy regions. In contrast to other generalized-ensemble

techniques the weight of the new ensemble is explicitly given by Eq. (3·11). One only needs to find an estimator for the ground-state energy E_0 which can be done by a procedure described in Ref. 14) and is much easier than the determination of weights for other generalized ensembles.

Another promising method to circumvent the need for determination of estimators for the weight is the replica-exchange method [16)–20)] (the method is also referred to as replica Monte Carlo method,[17)] multiple Markov chain method,[19)] and parallel tempering[20)]). The Monte Carlo (and molecular dynamics algorithms in dihedral space) in this generalized ensemble has been applied to an oligopeptide system.[21)] The details for the molecular dynamics algorithm (in Cartesian coordinates) have yet to be worked out, and it is the purpose of the present section to do so.[22)]

The generalized ensemble for replica-exchange method consists of M non-interacting replicas of the original system in the canonical ensemble at M different temperatures T_m ($m = 1, \cdots, M$). We arrange the replicas so that there is always exactly one replica at each temperature. Then there is a one-to-one correspondence between replicas and temperatures; the label i ($i = 1, \cdots, M$) for replicas is a permutation of the label m ($m = 1, \cdots, M$) for temperatures, and vice versa.

Let $X = \left(x_1^{[i(1)]}, \cdots, x_M^{[i(M)]}\right) = \left(x_{m(1)}^{[1]}, \cdots, x_{m(M)}^{[M]}\right)$ stand for a "state" in this generalized ensemble. Here, the superscript and the subscript in $x_m^{[i]}$ label the replica and the temperature, respectively.

Because the replicas are non-interacting, the weight factor for the state X in this generalized ensemble is given by the product of Boltzmann factors for each replica (or at each temperature):

$$W_{\mathrm{REM}}(X) = \exp\left\{-\sum_{i=1}^{M} \beta_{m(i)} H\left(q^{[i]}, p^{[i]}\right)\right\} = \exp\left\{-\sum_{m=1}^{M} \beta_m H\left(q^{[i(m)]}, p^{[i(m)]}\right)\right\} , \tag{3·12}$$

where $i(m)$ and $m(i)$ are the permutation functions.

We now consider exchanging a pair of replicas in the generalized ensemble. Suppose we exchange replicas i and j which are at temperatures T_m and T_n, respectively:

$$\begin{cases} x_m^{[i]} \equiv \left(q^{[i]}, p^{[i]}\right)_m & \longrightarrow \; x_m^{[j]\prime} \equiv \left(q^{[j]}, p^{[j]\prime}\right)_m , \\ x_n^{[j]} \equiv \left(q^{[j]}, p^{[j]}\right)_n & \longrightarrow \; x_n^{[i]\prime} \equiv \left(q^{[i]}, p^{[i]\prime}\right)_n , \end{cases} \tag{3·13}$$

where $p^{[i]\prime}$ and $p^{[j]\prime}$ are given by[22)]

$$\begin{cases} p^{[i]\prime} & \equiv \sqrt{\dfrac{T_n}{T_m}}\; p^{[i]} , \\ p^{[j]\prime} & \equiv \sqrt{\dfrac{T_m}{T_n}}\; p^{[j]} . \end{cases} \tag{3·14}$$

This assignment of momenta means that we just rescale uniformly the velocities of all the atoms in the replicas by the square root of the ratio of the two temperatures.

In order for this exchange process to converge towards an equilibrium distribution, it is sufficient to impose the detailed balance condition on the transition

probability $w(X \to X')$:

$$W_{\rm REM}(X)\, w(X \to X') = W_{\rm REM}(X')\, w(X' \to X)\ . \tag{3·15}$$

From Eqs. (3·12), (3·14) and (3·15), we have [22]

$$\begin{aligned}\frac{w(X \to X')}{w(X' \to X)} &= \exp\Big\{-\beta_m\left[K\left(p^{[j]\prime}\right)+E\left(q^{[j]}\right)\right]-\beta_n\left[K\left(p^{[i]\prime}\right)+E\left(q^{[i]}\right)\right] \\ &\qquad +\beta_m\left[K\left(p^{[i]}\right)+E\left(q^{[i]}\right)\right]+\beta_n\left[K\left(p^{[j]}\right)+E\left(q^{[j]}\right)\right]\Big\}\ , \\ &= \exp\Big\{-\beta_m\frac{T_m}{T_n}K\left(p^{[j]}\right)-\beta_n\frac{T_n}{T_m}K\left(p^{[i]}\right)+\beta_m K\left(p^{[i]}\right)+\beta_n K\left(p^{[j]}\right) \\ &\qquad -\beta_m\left[E\left(q^{[j]}\right)-E\left(q^{[i]}\right)\right]-\beta_n\left[E\left(q^{[i]}\right)-E\left(q^{[j]}\right)\right]\Big\}\ , \\ &= \exp\left(-\Delta\right)\ ,\end{aligned} \tag{3·16}$$

where $K(p)$ and $E(q)$ are, respectively, kinetic energy and potential energy, and

$$\Delta \equiv (\beta_n-\beta_m)\left(E\left(q^{[i]}\right)-E\left(q^{[j]}\right)\right)\ . \tag{3·17}$$

This can be satisfied, for instance, by the usual Metropolis criterion:

$$w(X \to X') \equiv w\left(x_m^{[i]} \;\middle|\; x_n^{[j]}\right) = \begin{cases} 1\ , & \text{for } \Delta \le 0\ , \\ \exp\left(-\Delta\right)\ , & \text{for } \Delta > 0\ . \end{cases} \tag{3·18}$$

Note that this is exactly the same criterion that was originally derived for Monte Carlo algorithm. [16)– 20)]

Without loss of generality we can assume $\beta_1 < \beta_2 < \cdots < \beta_M$. A simulation of the replica-exchange method [16)– 20)] is then realized by alternately performing the following two steps:

1. Each replica in canonical ensemble of the fixed temperature is simulated *simultaneously* and *independently* for a certain MC or MD steps.
2. A pair of replicas at neighboring temperatures, say $x_m^{[i]}$ and $x_{m+1}^{[j]}$, are exchanged with the probability $w\left(x_m^{[i]} \;\middle|\; x_{m+1}^{[j]}\right)$ in Eq. (3·18).

In the present approach, we employ molecular dynamics algorithm for Step 1.

The canonical expectation value of a physical quantity A at temperature T_m $(m = 1, \cdots, M)$ can be calculated by the usual arithmetic mean. For the expectation value at any intermediate temperature, we use the multiple-histogram reweighting techniques. [23)]

We are currently working on the further development of the replica-exchange method and applying the method to the protein folding (see the article by Y. Sugita in this volume) and the argon fluid (see the article by T. Nishikawa in this volume).

We remark that the exchanged quantity does not have to be temperature as long as it is in one-to-one correspondence with the replica (for instance, we can simulate a spin system of M replicas with M different magnetic field values). This is why we prefer the name replica-exchange method [16)] to parallel tempering [20)] (likewise, we prefer the term replica-exchange method to multiple Markov chain method, [19)] because molecular dynamics algorithm is also possible as described above).

§4. Results

We now present the results of our simulations based on simulated annealing and generalized-ensemble algorithms. All the simulations were started from randomly-generated conformations.

The first example is Met-enkephalin. This peptide consists of 5 amino acids with the amino-acid sequence: Tyr-Gly-Gly-Phe-Met. Because it is one of the smallest peptides, it has served as a bench mark for testing a new simulation method.

In Fig. 1 we display the time series of energy obtained by a Monte Carlo simulated annealing simulation. This run reaches the global minimum region ($E_P \leq -11$ kcal/mol), as the temperature is decreased during the simulation from 1000 K to 50 K. We also show the time series from a multicanonical Monte Carlo run in Fig. 2. It indeed exhibits a random walk in energy space between the lowest-energy region and very high energy region, escaping from states of energy local minima. Other generalized-ensemble algorithms exhibit similar random walks in energy space.[5),14)]

With generalized-ensemble algorithms, one can calculate various thermodynamic quantities as a function of temperature from a single simulation run by the reweighting techniques of Eq. (3·5). The results for average total conformational energy $\langle E_P \rangle_T$ and specific heat C from a production run of 1,000,000 MC sweeps are shown in Fig. 3. The results from the four generalized-ensemble algorithms discussed above (multicanonical, $1/k$ sampling, simulated tempering, and Tsallis) are superimposed in the figure.[5),14)] They agree with each other almost completely.

We have also studied the C-peptide, residues 1–13 of ribonuclease A. The amino-acid sequence is: Lys^+-Glu^--Thr-Ala-Ala-Ala-Lys^+-Phe-Glu-Arg^+-Gln-His+-Met. It is known from the X-ray diffraction data of the whole enzyme that the segment from Ala-4 to Gln-11 exhibits a nearly 3-turn α-helix.[24)] It was also found by CD[25)] and NMR[26)] experiments that the isolated C-peptide also has significant α-helix formation in aqueous solution at temperatures near 0 °C. The NMR experiment[26)] of the isolated C-peptide further observed the formation of the characteristic salt bridge between Glu-2^- and Arg-10^+ that exists in the native structure determined

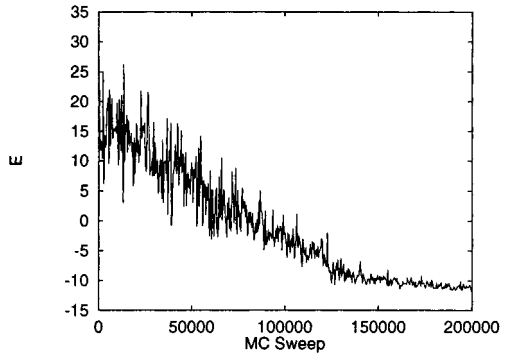

Fig. 1. Time series of energy E_P (kcal/mol) of Met-enkephalin from a Monte Carlo simulated annealing run.

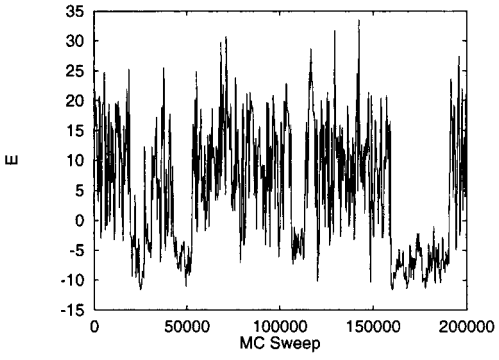

Fig. 2. Time series of energy E_P (kcal/mol) of Met-enkephalin from a multicanonical Monte Carlo run.

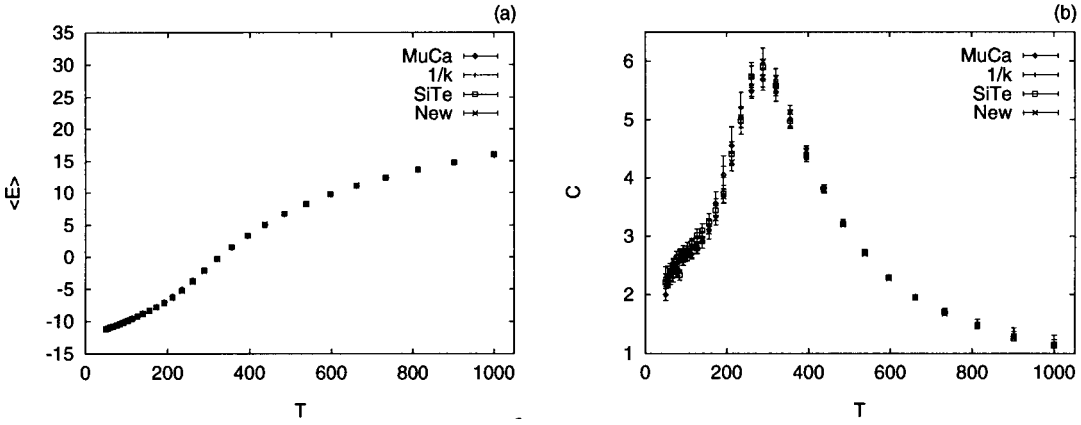

Fig. 3. Average total conformational energy $\langle E_P \rangle_T$ (kcal/mol) (a) and specific heat C (b) of Met-enkephalin as a function of temperature T (K). The results from four different generalized-ensemble Monte Carlo simulations are superimposed.

by the X-ray experiments of the whole protein.[24)]

In order to test whether our simulations can reproduce these experimental results, we first made 20 MC simulated annealing runs of 10,000 MC sweeps in gas phase.[27)] The temperature was decreased exponentially from 1000 K to 250 K for each run.

The lowest-energy conformation obtained exhibits an α-helix from Ala-5 to Gln-11, while the structure from the X-ray data has an α-helix from Ala-4 to Gln-11.[27)] The agreement of the backbone structures is conspicuous, but the side-chain structures are not quite similar. In particular, while the X-ray[24)] and NMR[26)] experiments imply the formation of the salt bridge between the side chains of Glu-2^- and Arg-10^+, the lowest-energy conformation obtained from the simulation does not have this salt bridge.

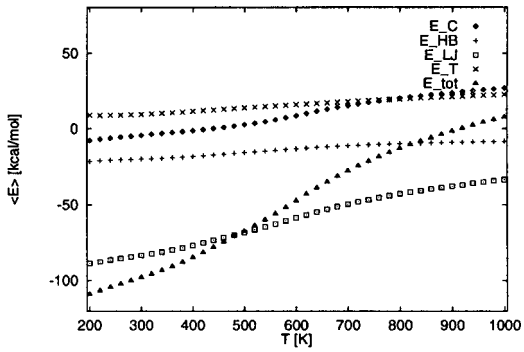

Fig. 4. Average potential energies $\langle E \rangle_T$ (kcal /mol) of C-peptide as a function of temperature T (K).

The disagreement is presumably caused by the lack of solvent in our simulations. We have therefore made multicanonical simulations of 1,000,000 MC sweeps for C-peptide with the inclusion of solvent effects by the distance-dependent dielectric function.[28)]

As emphasized above, the results from a single simulation run in multicanonical ensemble can be used to calculate various thermodynamic quantities as functions of temperature for a wide range of temperatures (see Eq. (3·5)). In Fig. 4 we plot the average total potential energy and each component as a function of temperature.

Among the component terms both electrostatic and Lennard-Jones terms vary most with the temperature. This is contrasted with our previous works on peptides with only electrically neutral side chains (Met-enkephalin [29] and homo-oligomers [30),31)]), where the changes of the Lennard-Jones term dominate that of the total potential energy. Hence, we understand that when some of the side chains are charged in the peptide, the contributions from the electrostatic interactions become a key factor in studying the peptide conformations (together with the Lennard-Jones term that is common in any peptide).

The lowest-energy conformation obtained has an α-helix from Ala-4 to Gln-11 and does have the characteristic salt bridge between Glu-2^- and Arg-10^+. This conformation and the corresponding X-ray structure are compared in Fig. 5. The figures were created with Molscript [32)] and Raster3D. [33)] The positions of the α-helix are identical for the two structures.

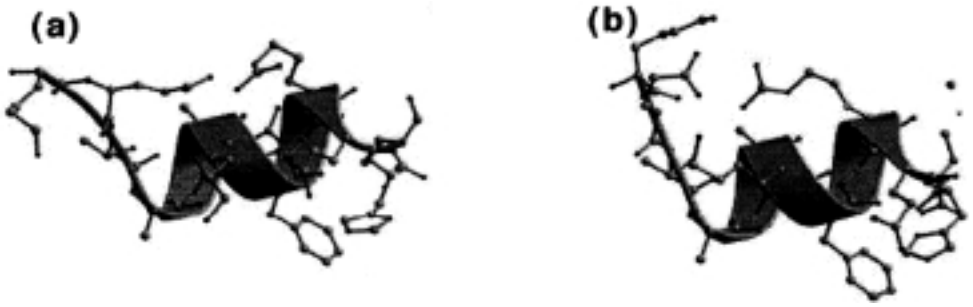

Fig. 5. X-ray structure of C-peptide (a) and the lowest-energy conformation of C-peptide obtained from a multicanonical Monte Carlo run in aqueous solution represented by the distance-dependent dielectric function (b).

§5. Conclusions

In this article, I have described the simulated annealing and generalized-ensemble algorithms for protein folding problem. The results of such simulations for C-peptide of ribonuclease A were in good agreement with various implications of experiments. I would like to emphasize again that the simulations were performed from randomly-generated initial conformations and that no structural information from experiments was used as input.

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