Prediction of Peptide Conformation by Multicanonical Algorithm: New Approach to the Multiple-Minima Problem

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We apply a recently developed method, the multicanonical algorithm, to the problem of tertiary structure prediction of peptides and proteins. As a simple example to test the effectiveness of the algorithm, met-enkephalin is studied and the ergodicity problem, or multiple-minima problem, is shown to be overcome by this algorithm. The lowest-energy conformation obtained agrees with that determined by other efficient methods such as Monte Carlo simulated annealing. The superiority of the present method to simulated annealing lies in the fact that the relationship to the canonical ensemble remains exactly controlled. Once the multicanonical parameters are determined, only one simulation run is necessary to obtain the lowest-energy conformation and further the results of this one run can be used to calculate various thermodynamic quantities at any temperature. The latter point is demonstrated by the calculation of the average potential energy and specific heat as functions of temperature.

INTRODUCTION

The prediction of tertiary structures of proteins from their primary structures remains one of the long-standing unsolved problems [for recent reviews, see, for example, refs. 1–4]. The problem amounts to finding the energy global minimum out of a huge number of local minima separated by high tunneling barriers. Within the presently available computer resources, traditional methods such as molecular dynamics and Monte Carlo simulations at experimentally relevant temperatures tend to get trapped in local minima, rendering the simulations strongly dependent on the initial conditions. One of the promising methods that alleviate this multiple-minima problem is simulated annealing.[5] The method is based on the "crystal forming" process; during simulation, temperature is lowered slowly from a sufficiently high temperature to a "freezing" temperature. Simulated annealing was used to refine protein structures from nuclear magnetic resonance (NMR) and X-ray data[6–8] and locate the global minimum-energy conformations of polypeptides and proteins.[9–11] The effectiveness of the method was further tested in many applications.[12–22] However, the algorithm is not completely free of faults. There is no established protocol for annealing and a certain number (which is not known *a priori*) of runs are necessary to evaluate the performance. Moreover, the relationship of the obtained conformations to the equilibrium canonical ensemble at a fixed temperature remains unclear.

A new powerful method, referred to as the multicanonical algorithm, was recently proposed by Berg et al.[23,24] The idea of this method is based on performing Monte Carlo simulations in a *multicanonical* ensemble[23,25] instead of the usual (canonical) Gibbs ensemble. The canonical distribution for *any* temperature can then be obtained from *one* multicanonical simulation run by the reweighting techniques.[26] In the multicanonical ensemble, all energies enter with equal probability so that a simulation may overcome the barriers between local minima (by connecting back to the high-temperature states). Because the multicanonical ensemble puts the energy on a 1-D random walk, the global-minimum state can be explored with ease. The method was originally developed to overcome the supercritical slowing down of first-order phase transitions,[24,27–29] but it has also been tested for systems with conflicting constraints such as spin glasses[30–32] and the 3-D random Ising model.[33] The latter systems suffer from a similar multiple-minima problem and it was claimed that the multicanonical algorithm outperforms simulated annealing in these cases.[30]

In the present work, we apply the multicanonical algorithm to the problem of tertiary structure prediction of peptides and proteins. Because the purpose of this work is primarily to test the effective-

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ness of the algorithm, we have studied one of the simplest peptides, met-enkephalin. This peptide is convenient for our purpose because the lowest-energy conformation for the potential energy function ECEPP/2[34–36] is known[37,38] and analyses with Monte Carlo simulated annealing with ECEPP/2 also exist.[18,21] We shall show that by running the multicanonical simulation only once we can not only reproduce the lowest-energy conformation but also obtain the canonical distribution at various temperatures.

METHODS

Potential Energy Function

Met-enkephalin has the amino-acid sequence Tyr-Gly-Gly-Phe-Met. For our simulations, the backbone was terminated by a neutral NH_2-group at the N-terminus and a neutral-COOH group at the C-terminus as in the previous works of met-enkephalin.[10,18,21,37,38] 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 parameters for the energy function were adopted from ECEPP/2,[34–36] and the computer code KONF90,[15,16] which is based on the Metropolis algorithm,[39] was modified to accommodate the multicanonical method. The peptide bond dihedral angles ω were fixed at the value 180° for simplicity, which leaves 19 dihedral angles as independent variables.

Multicanonical Algorithms

Because the multicanonical algorithm is already described in detail elsewhere,[23] we give only a short overview in this subsection. In the canonical ensemble, configurations at an inverse temperature $\hat{\beta} \equiv 1/RT$ are weighted with the Boltzmann factor

$$\mathcal{P}_B(E) = \exp(-\hat{\beta}E) \tag{1}$$

The resulting probability distribution is given by

$$P_B(E) \propto n(E)\mathcal{P}_B(E) \tag{2}$$

where $n(E)$ is the spectral density. Because $n(E)$ is a rapidly increasing function and the Boltzmann factor decreases exponentially, $P_B(E)$ generally has a bell-like shape. At a finite temperature, the value of $P_B(E)$ for low E is smaller by many orders of magnitudes than the maximum value of $P_B(E)$ (see Fig. 1).

In the *multicanonical* ensemble,[23,25] on the other hand, the probability distribution is defined in such a way that a configuration with any energy enters with equal probability:

$$P_{mu}(E) \propto n(E)\mathcal{P}_{mu}(E) = \text{const} \tag{3}$$

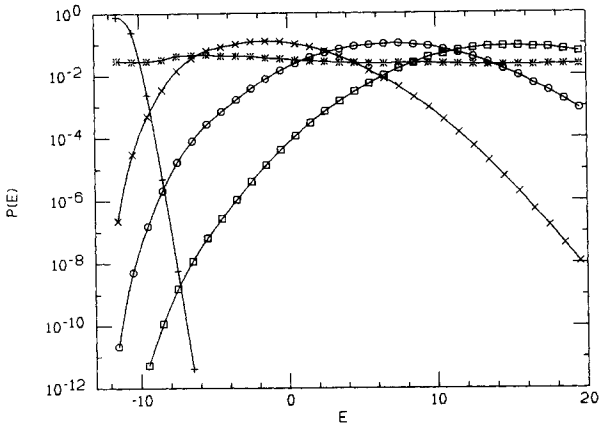

Figure 1. Probability distributions of multicanonical ensemble (*) and canonical ensembles at T = 50 K (+), 300 K (×), 500 K (○), and 1000 K (□) for met-enkephalin.

It then follows that the multicanonical weight factor should have the form

$$\mathcal{P}_{mu}(E) \propto n^{-1}(E) \tag{4}$$

To define the explicit form of this weight factor, we introduce two parameters $\alpha(E)$ and $\beta(E)$ as follows[23,24]:

$$\mathcal{P}_{mu}(E) \equiv e^{-B(E)} = \exp\{-(\hat{\beta} + \beta(E))E - \alpha(E)\} \tag{5}$$

Note that for any fixed $\beta(E)$ and $\alpha(E)$, this leads to the canonical weight factor with the inverse temperature $\beta = \hat{\beta} + \beta(E)$, therefore the name "multicanonical." From eqs. (4) and (5), we have

$$e^{-\beta(E)E-\alpha(E)} \propto P_B^{-1} \tag{6}$$

Here, the argument appears to be circular because we do not know $P_B(E)$ *a priori*. In fact, it is not. For a numerical simulation, it is sufficient to find estimators for the multicanonical parameters $\beta(E)$ and $\alpha(E)$. These can be obtained by an iterative procedure that will be introduced in the next subsection. Once the parameters are determined, one multicanonical run is in principle enough to find the global minimum and calculate all thermodynamic quantities by reweighting.[26]

The standard Markov process (for instance, in a Metropolis update scheme[39]) is well suited to generate configurations that are in equilibrium with respect to the multicanonical distribution. Because in the multicanonical ensemble all energies have equal weight, the energy is forced onto a 1-D random walk (when simulated with local updates), which ensures that the system can overcome any energy barrier.

Implementation of the Algorithm

In an actual simulation, the parameters $\alpha(E)$ and $\beta(E)$ can be determined as follows. We first run a canonical Monte Carlo simulation at a sufficiently high temperature $\hat{\beta}_0^{-1}$. We approximate $P_B(\hat{\beta}_0, E)$ at

this temperature by a histogram $\tilde{P}(\hat{\beta}_0, E_i)$ ($i = 1, \ldots, N$) where N is the number of energy bins. We then determine the mode, E_{max}, of the histogram, where the histogram has its maximum. We restrict ourselves to the energy range $E \leq E_{max}$, setting $\beta(E) = 0$ and $\alpha(E) = 0$ outside of this range. By eq. (6), we have

$$-\beta(E_i)E_i - \alpha(E_i) = \ln(\tilde{P}^{-1}(\hat{\beta}_0, E_i)) + \text{const.} \equiv y_i \quad (7)$$

The parameters $\alpha(E_i)$ and $\beta(E_i)$ can now be obtained, for example, by connecting two adjacent points (E_i, y_i) and (E_{i+1}, y_{i+1}) by a straight line [$-\beta(E_i)$ being the slope of the line]. Because the statistics of $\tilde{P}$ are poor for low energies, this procedure should, in general, be iterated a few times. By labeling the iteration number by k, the probability distribution $\tilde{P}^k(\hat{\beta}_0, E_i)$ of the kth iteration is calculated from a Monte Carlo run with the weight factor of (5), where the parameters $\alpha^{k-1}(E_i)$ and $\beta^{k-1}(E_i)$ which were determined in the previous iteration and $\hat{\beta} = \hat{\beta}_0$ are used in (5). Note that the transition probability $w(E \rightarrow E')$ for Metropolis criterion is now given by

$$w(E \rightarrow E') = 1, \quad \text{if } \Delta \equiv B(E') - B(E) \leq 0$$
$$= e^{-\Delta}, \quad \text{if } \Delta > 0 \quad (8)$$

where $B(E)$ is defined in (5). Once $\tilde{P}^k(\hat{\beta}_0, E_i)$ is given, the parameters $\alpha^k(E_i)$ and $\beta^k(E_i)$ are obtained from (7). Note that with this procedure the probability distribution becomes flatter and the region with lower energy is explored as the iteration number is increased. This iterative procedure is terminated when the obtained probability distribution becomes reasonably flat in the chosen energy range. Further, near the ground-state energy we expect to see this flat distribution drop to zero abruptly in a step function-like behavior. This is the criterion for the optimal choice of $\alpha(E)$ and $\beta(E)$. After determination of $\alpha(E)$ and $\beta(E)$, we make one long production run. From this production run, one can not only locate the global-energy minimum but also obtain the canonical distribution at any temperature $\hat{\beta}^{-1}$ for all $\hat{\beta} \geq \hat{\beta}_0$.[31] The latter is done by the reweighting techniques[26] as follows:

$$P_B(\hat{\beta}, E) = \frac{e^{B(E) - \hat{\beta}E} P_{mu}(E)}{\sum_E e^{B(E) - \hat{\beta}E} P_{mm}(E)} \quad (9)$$

For our study of met-enkephalin, we first made a preliminary canonical simulation at $T = 1000$ K with 10^4 Monte Carlo steps. We iterated this process four times to determine optimal $\alpha(E)$ and $\beta(E)$. We then made one production run with 10^5 Monte Carlo steps, recording the time series of the energy and the torsion angles. The CPU time for the production run was ≈370 min on an IBM RS/6000 [320H] workstation (equivalent to ≈10 min on HITAC S820/80) and accordingly that for the four preliminary runs was 40% of the production run.

RESULTS

Average Energy and Specific Heat

We analyze the results of the production run by first calculating the (canonical) probability distributions, average energy, and specific heat at various temperatures.

In Figure 1, we show the multicanonical probability distribution $P_{mu}(E)$ together with the canonical distributions $P_B(E)$ at $T = 50$, 300, 500, and 1000K. These P_Bs were obtained from P_{mu} by the reweighting of (9). Note that P_{mu} is nearly flat (at least of the same order) throughout the whole energy range, while P_Bs do vary many orders of magnitude as a function of energy. In particular, at higher temperatures ($T = 500$ and 1000K), where energy barriers can be easily overcome, it would require canonical simulations at least 10^{10} more simulation time than multicanonical algorithm to explore the global-minimum energy region with the same quality of statistics. This clearly illustrates the advantage of the multicanonical method over the canonical Monte Carlo simulations at a fixed temperature.

In Figure 2, we show the average energy as a function of temperature. This was again obtained by the reweighting of (9). The values vary smoothly over the whole temperature range. To roughly estimate the errors of our data, we divided our time series into two bins, the first half and the second half of 10^5 Monte Carlo steps. We calculated the averages separately for both bins and took their difference as an estimate for the error, which we included (for certain temperatures) in the figure. The value ≈ −12 kcal/mol at $T = 50$ K is close to the global-minimum

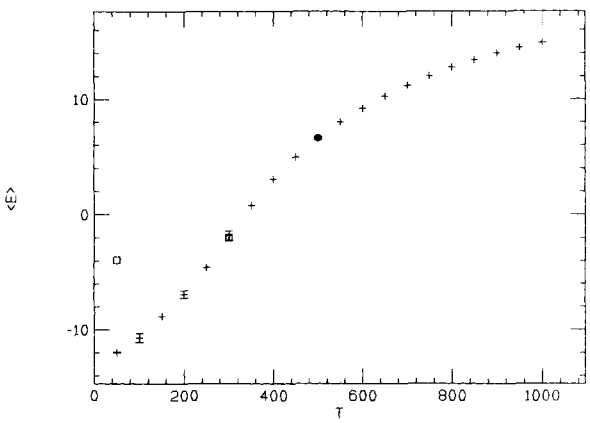

Figure 2. Average energy of met-enkephalin as a function of temperature evaluated by the multicanonical algorithm. The results of canonical simulations at fixed temperatures (50 and 300 K) are also plotted (□).

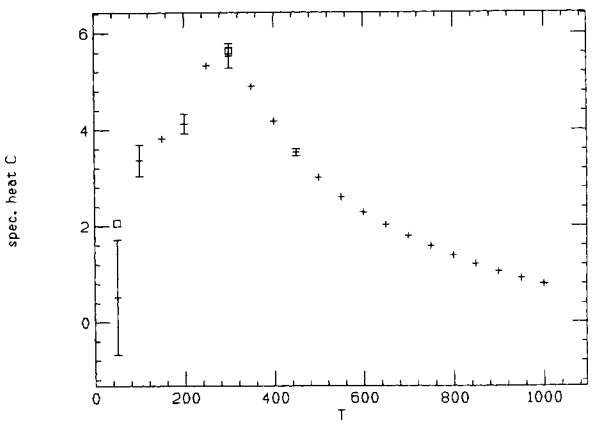

Figure 3. Specific heat of met-enkephalin as a function of temperature evaluated by the multicanonical algorithm. The results of canonical simulations at fixed temperatures (50 and 300 K) are also plotted (□).

energy obtained by other methods.[18,21,37,38] This indicates that the multicanonical algorithm avoids being trapped in a local-energy minimum. To illustrate the effectiveness of the algorithm, we have also listed in the figure the values obtained from fixed-temperature canonical simulations with 10^5 Monte Carlo steps at $T = 50$ and 300 K. Note that the value for $T = 50$ K is completely off from the multicanonical result, indicating that this canonical run got trapped in a local minimum. The value at $T = 300$ K is in agreement with the multicanonical run. In fact, this kind of analysis will tell us how many Monte Carlo steps are necessary in order that a usual canonical simulation at a certain temperature may be trusted.

In Figure 3, we likewise present the "specific heat" (per residue), which is defined by

$$C = \beta^2 \frac{\langle E^2 \rangle - \langle E \rangle^2}{5} \tag{10}$$

It has a peak around $T = 300$ K, which indicates that this temperature is important for peptide folding. The result agrees with the previous evaluation from canonical simulations at several temperatures.[40] The results from the canonical simulations at $T = 50$ and 300 K also agree roughly with the multicanonical results. This indicates that energy fluctuations are not much different whether we do simulations in the entire conformational space or around a local minimum.

Lowest-Energy Conformation

During the production run, the system reached the global-energy minimum region in six separate short time spans. The lowest-energy conformation within each visit is listed in Table I together with the global-minimum energy conformation (conformation A in Table I) obtained by simulated annealing.[21] Conformation A has essentially the same structure as the global-minimum conformation obtained by another method.[37] The small differences presumably arise because the peptide bond dihedral angles ω were fixed at the value 180° in ref. 21 while they were allowed to vary in ref. 37. Because we use the same computer code, KONF90,[15,16] as in ref. 21 and fix ω at 180° in this work, we compared the present simulations with the global-minimum conformation of

Table I. Energy and dihedral angles of the lowest-energy conformations of met-enkephalin obtained by a multicanonical run.

	Conformation						
	A	1	2	3	4	5	6
E (kcal/mol)	−11.9	−11.9	−12.0	−12.0	−12.1	−12.0	−11.9
ϕ_1	98	90	91	90	97	96	98
ψ_1	154	153	152	154	151	153	156
ϕ_2	−161	−160	−157	−161	−158	−161	−163
ψ_2	69	72	64	71	71	68	65
ϕ_3	65	64	66	63	64	64	66
ψ_3	−93	−95	−92	−95	−94	−89	−92
ϕ_4	−85	−82	−80	−77	−83	−85	−80
ψ_4	−27	−26	−29	−32	−30	−31	−29
ϕ_5	−83	−81	−82	−78	−80	−82	−86
ψ_5	142	142	138	137	145	151	147
χ_1^1	−179	179	−177	179	179	−178	−176
χ_1^2	−112	−110	−117	−109	−111	−115	−114
χ_1^3	149	144	146	143	149	145	142
χ_4^1	180	−176	178	177	180	−178	180
χ_4^2	73	79	81	86	79	78	78
χ_5^1	−65	−64	−67	−67	−66	−67	−66
χ_5^2	180	−179	180	180	−176	180	176
χ_5^3	179	178	179	−179	−179	−178	−178
χ_5^4	−55	−66	−59	−62	−61	−60	−57

Conformation A is the lowest-energy conformation obtained by Monte Carlo simulated annealing (taken from ref. 21).

ref. 21 (conformation A in Table I). We remark that by fixing the ω angles to the values of ref. 37 we were able to reproduce the same structure as in ref. 37.

In Table I, conformations 1–6 are the results at Monte Carlo steps 20,128, 39,521, 44,462, 65,412, 89,413, and 95,143. Hence, the system reached the lowest-energy region in every 5,000–20,000 Monte Carlo steps. The energies are almost all equal, and the lowest-energy value in the present work (− 12.1 kcal/mol) is slightly less than the previous result (− 11.9 kcal/mol) by simulated annealing.[21] Most of the dihedral angles of the six conformations also agree with the corresponding ones of conformation A within ≈5°. Hence, the conformations in Table I are all equivalent. Note that these six conformations were obtained by only one production run of multicanonical simulation, while conformation A was one of 40 Monte Carlo simulated annealing runs (with 10^4 Monte Carlo steps). In this respect, the multicanonical algorithm is superior to simulated annealing; only one run is required for the former, whereas in the latter one does not know *a priori* how many runs are required and the convergence must be tested by running at least several times.

By utilizing the reweighting of (9), we have calculated the fraction in which the lowest-energy conformation exists at various temperatures (50, 100, 200, 300, and 500 K). For this, we consider that a conformation is of the lowest-energy structure if all 19 dihedral angles agree with those of conformation A in Table I within ±20°. The results are shown in Figure 4. As expected, at T = 50 K the peptide is almost always in "ground state." As the temperature rises, the conformation is thermally excited and the fraction in Figure 4 decreases. However, at T = 300 K the peptide still stays close to the ground state for a substantial amount of time (≈35%). This kind of analysis will be useful in understanding the relation between the conformation with the global-minimum potential energy and the native conformation around room temperature.

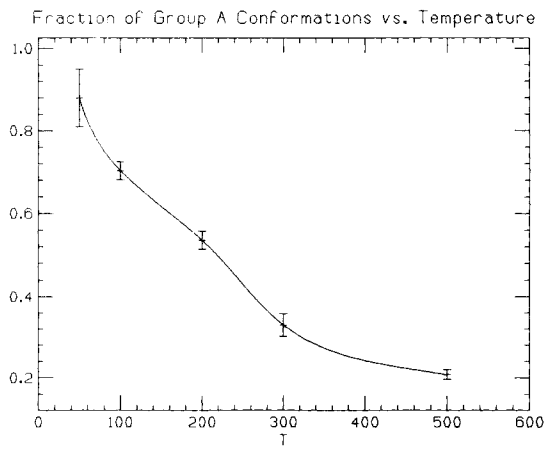

Figure 4. Fraction of the occurrence of the lowest-energy structure of met-enkephalin as a function of temperature.

CONCLUSIONS AND DISCUSSION

In this article, we have applied the recently developed multicanonical algorithm to the problem of peptide conformation prediction. This method avoids getting trapped in a local minimum of energy function by connecting back to high-temperature states and enhances in this way the probability of finding the global minimum. This property is exactly what we need for peptide structure prediction. We have demonstrated the effectiveness of the algorithm by reproducing the lowest-energy conformation of met-enkephalin. This was achieved by only one production run of simulation, whereas another powerful method for overcoming energy barriers such as simulated annealing usually requires many more runs to confirm the results. Further, the multicanonical algorithm can yield various thermodynamic quantities as a function of temperature from only one production run. This was not possible by previous methods. To illustrate this property, we have calculated the average energy and specific heat at various temperatures.

Although our method for the determination of the multicanonical parameters $\alpha(E)$ and $\beta(E)$ is general, it required about 50% of the CPU time spent for the production run. It is thus desirable to develop a more efficient method for the determination of these parameters. Work in this direction is in progress.

The CPU time required for simulations by KONF90 with a fixed number of Monte Carlo steps scales like $\mathcal{O}(N^{2.2})$ on a vector machine, where N is the number of residues (data not shown). As the system size increases, the required number of Monte Carlo steps also increases to cover the relevant energy range. Judging from our expriences with the spin glass systems, our estimate for the scaling factor of the total CPU time of the present algorithm is at most $\mathcal{O}(N^3)$. Hence, the prediction of tertiary structure of a small protein such as BPTI seems within our reach.

Finally, as far as one is only interested in finding the global minimum, another algorithm, *random cost optimization*,[41] that is related to the present method is also promising. Comparison of the performance of the multicanonical algorithm and random cost optimization in the problem of peptide structure prediction is now underway.

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