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CAMDAS: An automated conformational analysis system using molecular dynamics

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Summary

We present an automated conformational analysis program, CAMDAS (Conformational Analyzer with Molecular Dynamics And Sampling). CAMDAS performs molecular dynamics (MD) calculations for a target molecule and samples conformers from the trajectory of the MD. The program then evaluates the similarities between each of the sampled conformers in terms of the root-mean-square deviations of the atomic positions, clusters similar conformers, and finally prints out the clustered conformers. This MD-based conformational analysis is a broadly used method, and CAMDAS is intended to provide a convenient framework for the method. CAMDAS has the ability to find the representative conformers automatically from an arbitrarily given structure of the molecule. The accuracy of the program was examined using *N*-acetylalanine-*N′*-methylamide, and the obtained result was consistent with that of the systematic search method. In the test calculation of cyclodecane, CAMDAS could identify most of the known conformers and their conformational enantiomers by examining only 5000 conformers. In addition, the potential-scaled method, which we have developed previously as an accelerating technique for MD, could find two additional conformers of cyclodecane that have not been reported. CAMDAS presents a convenient way to find the energetically possible conformers of a molecule, which is needed especially in the early stage of drug design.

Introduction

Conformational analysis of pharmaceutical molecules is becoming more important in the field of drug design. The aim of conformational analysis is to find the representative conformers which can be adopted by a target molecule. The information obtained from conformational analysis can be used in a successive drug-design process, such as (quantitative) structure–activity relationship studies or pharmacophore modeling. Therefore, if a convenient and efficient tool for conformational analysis is available, it would be useful for the iterative process of drug design.

There are numerous methods [1–13] available for conformational analysis. Generally, they are divided into three groups: (i) systematic search method [5–7,11] that generates conformers by varying the degrees of freedom (typically dihedral angles) exhaustively and evaluates the potential energy of each conformer; (ii) Monte Carlo method (MC) [2,3] that samples the conformational space in a random way; and (iii) molecular dynamics method (MD) [1,4,8–10,12,13] that solves Newton's equation of motion and searches the conformational space by the kinetic energy of the molecule. Among these methods, MD would have several advantages. In MD calculation, the molecule moves along the gradient of its potential surface. Moreover, the kinetic energy in the system, which is a function of the temperature, allows it to surmount energy barriers on the potential surface [14]. This means that MD can preponderantly search important, low-energy regions in the conformational space, while the other two methods search the space uniformly or randomly. There are several methods that enhance the ability of MD to search the conformational space. A well-known tech-

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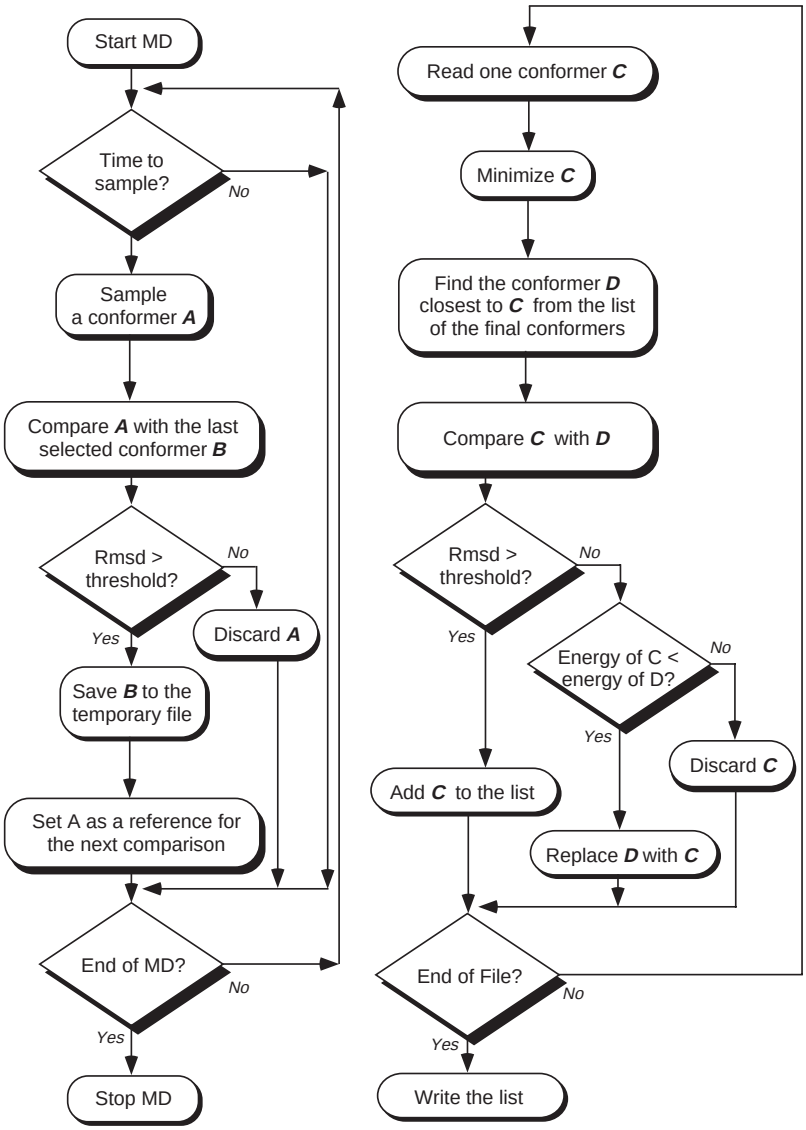

Fig. 1. Flow chart of the calculation of CAMDAS. The left and right columns indicate the calculation schemes in the molecular dynamics and the clustering stages, respectively.

nique to accelerate MD is high-temperature MD [1], in which a very high temperature gives higher kinetic energy to the molecule. In addition, we have previously developed another accelerating method [8,9]. This method, namely 'potential-scaled MD', scales down the potential energy involved in the desired degrees of freedom. Our potential-scaled method is based on the fact that both raising the temperature and decreasing the potential energy have the same effect on the Boltzmann factor, which governs the population of conformers. In the previous report [8], we have proven that the potential-scaled MD could improve the efficiency of the conformational search, as the high-temperature method did.

Another advantage of MD in conformational analysis is its simplicity. For example, MD can be easily applied to a cyclic structure, to which the systematic search method is difficult to apply without any special effort. Although there are some modified systematic search techniques to overcome this problem of cyclic molecules, such as a method breaking some bonds in the ring system [7], difficulties would still arise in the treatment of more complex structures. In contrast, the conformational analysis using MD is very easy. Even when the target molecule has a fairly complex structure, all we need to do is to provide an arbitrary, initial structure. The molecule then changes its structure by its kinetic energy and visits its low-energy conformational states spontaneously. The MD-based conformational analysis has been successfully applied to various systems, including some cyclic compounds [12,13].

As mentioned above, the MD-based conformational analysis is quite simple in principle. However, it still needs some tedious operations in actual use. First, one

must sample thousands of conformers of the molecule from the trajectory of the MD calculation. In general, these conformers are not in the energy minima on the potential surface. Therefore, one should minimize each of these conformers one by one. Finally, one should compare and cluster these conformers by some criteria. If an integrated computer program is available that performs these processes automatically, the MD-based conformational analysis would become more convenient in practical use. In this paper, we present such an automated conformational analysis program, CAMDAS (Conformational Analyzer with Molecular Dynamics And Sampling). CAMDAS performs an MD calculation for a target molecule and samples conformers appearing during the MD. CAMDAS then evaluates the similarities between each of the sampled conformers in terms of the root-mean-square deviations (rmsd), clusters similar conformers, and finally prints out the clustered conformers of the molecule. In this way, CAMDAS can find the representative conformers automatically from an arbitrarily given structure of the molecule. In the following section, we describe the detailed features of CAMDAS. Some examples are also given. We have also implemented the potential-scaled MD in CAMDAS and have examined its usefulness in the course of the following test calculations.

Methods

Molecular dynamics and sampling stage

The calculation scheme used by CAMDAS is shown in Fig. 1. At first, CAMDAS performs an MD calculation to explore the conformational space of the target molecule. During the calculation, CAMDAS samples conformers appearing on the trajectory at every user-defined time interval. In this sampling stage, CAMDAS roughly clusters the similar conformers in the following way. When a conformer is sampled, CAMDAS superposes it on the last selected one and calculates the rmsd of the corresponding atoms between the two conformers. If the value of rmsd is smaller than the threshold, the current conformer is thought to be the same as the last one and is rejected. Otherwise, the sampled conformer is accepted. In the latter case CAMDAS writes the last accepted conformer to a temporary file and the current conformer will be used as the reference at the next sampling time. These procedures ensure that CAMDAS should sample the conformers only when the conformational transition occurs in the MD calculation. This would reduce the number of conformers sampled by CAMDAS and the computational time required in the next calculation stage.

Minimization and clustering stage

When the MD calculation has been done, the conformers sampled during the MD have been stored in a temporary file. As described above, CAMDAS has already eliminated the similar conformers at the sampling time. However, this selection has compared the sampled conformer only with the most recently accepted one. Thus, conformers close to each other might exist in the file if the molecule has visited the same energy minimum repeatedly during the MD run. Therefore, reclustering is needed to extract the unique and representative conformers of the molecule. CAMDAS does this in the following way. At first CAMDAS reads one conformer from the temporary file and minimizes it. If this is not the first cycle of the reclustering stage, CAMDAS has the list of conformers that have been finally accepted. If this is the case, CAMDAS then superposes the current, minimized conformer on all of the accepted ones sequentially and calculates the rmsd for each pair. After this, the conformer in the accepted list that gives the smallest rmsd value is compared with the current conformer. If the rmsd value is smaller than the threshold, these two conformers are considered to be the same. In this case, a conformer that has lower potential energy is selected and the other is discarded. Otherwise, the current conformer is considered as a new one and added to the finally accepted conformers' list. CAMDAS repeatedly executes these operations for reclustering until it reads all of the conformers in the temporary file. When these calculations have been done, the list should include the conformers that are distinct from each other at least by the rmsd threshold. CAMDAS finally sorts the list by energy and writes out the conformers on the list.

Force field used in CAMDAS

CAMDAS uses Allinger's MM2 force field [15] to evaluate the potential energy surface of the molecule. The current version of CAMDAS is intended for use with a molecular modeling program, CAChe (Oxford Molecular Group, Oxford, U.K.). CAChe has a handy graphical interface for building the three-dimensional structures of molecules. It is able to write out the built structure in Protein Databank (PDB) file format. In addition, CAChe can automatically assign MM2 force field parameters to the molecule and write out their list in a plain text format. CAMDAS reads these PDB and parameter files directly and uses them in the calculations described above. Although CAMDAS itself does not have any graphical interface to communicate with the user, this feature of collaboration with CAChe would prevent the user from doing boring tasks such as writing a Z-matrix or parameter assignment by hand. Other implementations of CAMDAS that communicate with some other modeling programs are now under consideration.

Calculation keywords

CAMDAS has many calculation options that control its run. Each option is specified by a 'keyword', a simple string which should be described in a control file. In the

TABLE 1
LIST OF CALCULATION KEYWORDS

Conformation sampling	
NCONF	Specifies the maximum number of conformers
RMSMAT	Specifies rmsd threshold
NMATCH	Specifies atoms used in superposition
RMSCALC	Specifies atoms used in rmsd calculation
NSAMPLE	Specifies the time interval for sampling
Molecular dynamics	
MDSTEP	Specifies a period of MD calculation
TEMP0	Specifies a reference temperature
NVT	Performs MD at constant temperature
SHAKE	Fixes the bond lengths during MD
Minimization	
MINSTEP	Specifies maximum steps of minimization
DRMS	Specifies a criterion for convergence by rms of gradients
DENE	Specifies a criterion for convergence by energy reduction per step
Force field	
WTANG	Specifies a scaling factor for the angle term during MD
WTTOR	Specifies a scaling factor for the torsional term during MD
Constraints	
DCON	Specifies distance constraints
ACON	Specifies angle constraints
TCON	Specifies dihedral constraints
BELLY	Fixes a part of the system

beginning of the run, CAMDAS reads these keywords written in the control file and determines how to do the calculation. The major keywords used by CAMDAS are listed in Table 1. Here are brief explanations for them.

NMATCH and RMSCALC are important keywords that determine how to cluster conformers. NMATCH specifies which atoms in the molecule are considered in superposing conformers. RMSCALC specifies atoms to be included in the rmsd calculation. Because CAMDAS clusters conformers in terms of the rmsd between them, RMSCALC can be used for specifying an interesting part of the molecule in the conformational analysis. For example, when the target molecule is a peptide, one may want to classify conformers by the deviations of the main chain. In this case, both NMATCH and RMSCALC should be set to atoms of the main chain. This leads CAMDAS to superpose conformers about the main chain and cluster them by the deviations of the chain. On the other hand, when the target molecule has a rigid region (e.g., some aromatic rings) in which the conformational change would rarely occur, NMATCH may specify atoms in this region. In this case, other interesting parts (e.g., a functional group attached to the aromatic ring) can be specified through RMSCALC. This would reveal the conformational behavior of the molecule more clearly.

MD calculations can be performed under various conditions in CAMDAS. Keywords NVT and TEMP0 control the temperature of the calculation system using Berendsen's weak coupling algorithm [16]. Giving a high value (typically 600–1200 K) to TEMP0 could enhance the ability of the molecule to surmount the potential energy barriers, because the kinetic energy of each atom would be increased at high temperature. It is a general method to accelerate the conformational search using MD [1]. Keywords WTANG and WTTOR are provided for the use of the potential-scaled MD [8]. WTANG and WTTOR, which take values from 0 to 1, are weighting factors of the bond-bending and torsional terms in the force field, respectively. The energy heights of these terms can be reduced by setting the keywords to values smaller than 1. Because the torsional term is one of the major components of the potential energy barriers, reducing it would make conformational transitions easier. Decreasing the bond-bending term causes a molecule to be more flexible and would help the molecule to pass through alternative paths in surmounting the energy barriers.

Keywords DCON, ACON, and TCON set constraints for distances, bond angles, and torsional angles in a molecule, respectively. These keywords specify a force constant and upper and lower bounds of a target value of each constraint. These kinds of constraints would be useful especially when some experimental information, such as distances or torsional angles derived from nuclear Overhauser effects or coupling constants of NMR spectroscopy, is available.

Examples for the applications of CAMDAS

We show two applications of CAMDAS hereafter. One is the conformational analysis of a dipeptide mimic, *N*-acetylalanine-*N′*-methylamide (AAMA, Fig. 2). The conformational space of AAMA is mainly defined by two backbone dihedrals, ϕ and ψ, so the potential energy surface of the molecule can be clearly investigated by a systematic search around these dihedrals. In comparison with the result from the systematic search, we have checked whether CAMDAS could generate the correct, representative conformers of the molecule.

Another application is an analysis of cyclodecane. This is an example for the calculation of a cyclic structure. For cyclodecane, Lipton and co-workers have studied its conformational property using their systematic search program [7]. They have reported that there were 17 uni-

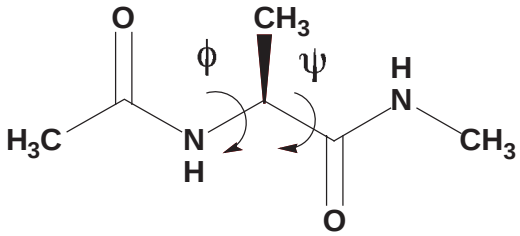

Fig. 2. Chemical structure of AAMA. Round arrows indicate ϕ and ψ dihedral angles.

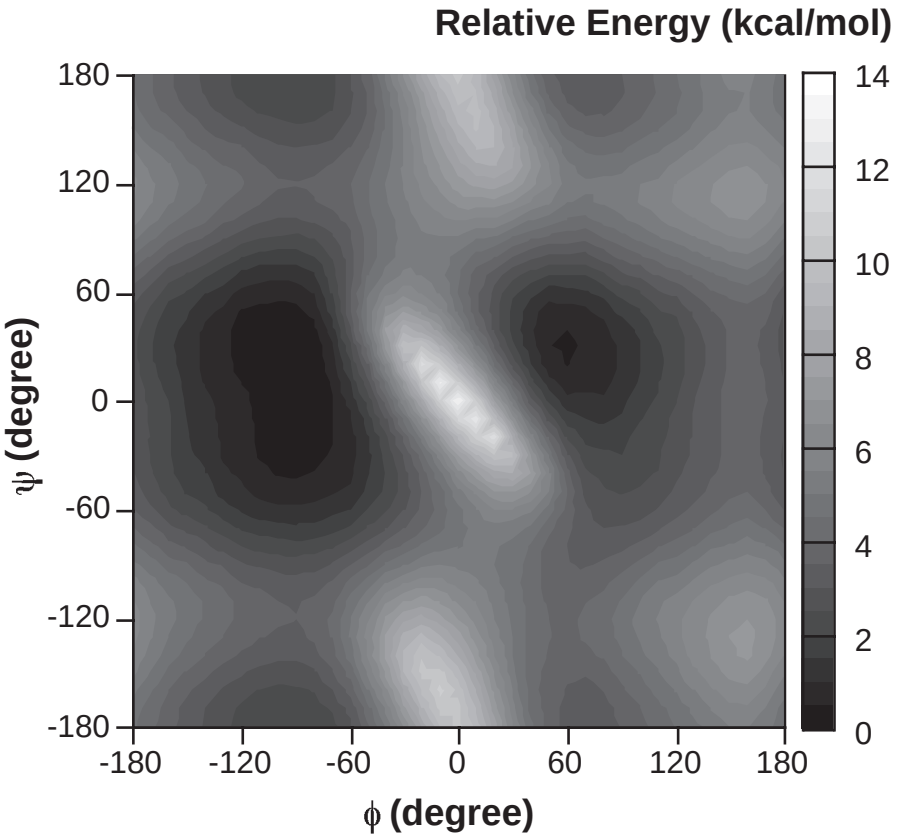

Fig. 3. Potential energy surface of AAMA. Each contour represents a relative energy from the most stable region.

que conformers of cyclodecane. Using their results as a reference, we have tested whether CAMDAS could find these conformers of cyclodecane.

Test for AAMA At first, the location of the energy minima on the conformational space of AAMA was investigated using the energy minimization feature of CAMDAS. Upon the calculation, each of the ϕ and ψ torsional angles was rotated successively by 10°. For each conformation, its potential energy was obtained after minimization. The minimization was carried out until the root mean square of the gradients of the potential energy was below 0.05 kcal $mol^{-1}Å^{-1}$. Values of ϕ and ψ were restrained with a force constant of 5000 kcal mol^{-1} rad^{-4}, and the other degrees of freedom were free to move during the minimization. The electrostatic interactions were considered as bond dipole–dipole interactions with a dielectric constant of 80, for mimicry of the solvent water.

An MD calculation for sampling was performed for 200 ps with an integral time step of 1 fs. The temperature of the system was maintained at 1200 K, and the lengths of the covalent bonds were fixed with the SHAKE algorithm [17] through the MD. Conformers were sampled at every 100 steps (0.1 ps) and preclustered with an rmsd threshold of 0.1 Å during the MD. Values of rmsd were calculated about the atoms of the peptide backbone. After the MD, the reclustering of the sampled conformers was performed with an rmsd threshold of 0.3 Å. Before the clustering, each conformer was minimized until the root mean square of the gradients of the potential energy was below 0.05 kcal $mol^{-1}Å^{-1}$. Again, the electrostatic interactions were represented as bond dipole–dipole interactions with a dielectric constant of 80.

Test for cyclodecane For cyclodecane, we performed two calculations: one with the normal potential energy function and another in which the angle and torsional terms of the potential were reduced to one-half their original values (WTANG = WTTOR = 0.5, see above). The latter calculation was performed to examine how this potential-scaled method would affect the efficiency of CAMDAS. The other calculation conditions were exactly the same between the two calculations. MD calculations for sampling were performed for 500 ps with an integral time step of 1 fs. The temperature of the system was maintained at 1200 K and the lengths of the covalent bonds were fixed with the SHAKE algorithm through the MD. Conformers were sampled at every 100 steps (0.1 ps) and preclustered with an rmsd threshold of 0.1 Å during the MD. The superposition and rmsd calculation were done for all of the carbon atoms. After the MD, the reclustering of the sampled conformers was performed with an rmsd threshold of 0.2 Å. Before the clustering, each conformer was minimized until the root mean square of the gradients of the potential energy was below 0.001 kcal $mol^{-1}Å^{-1}$.

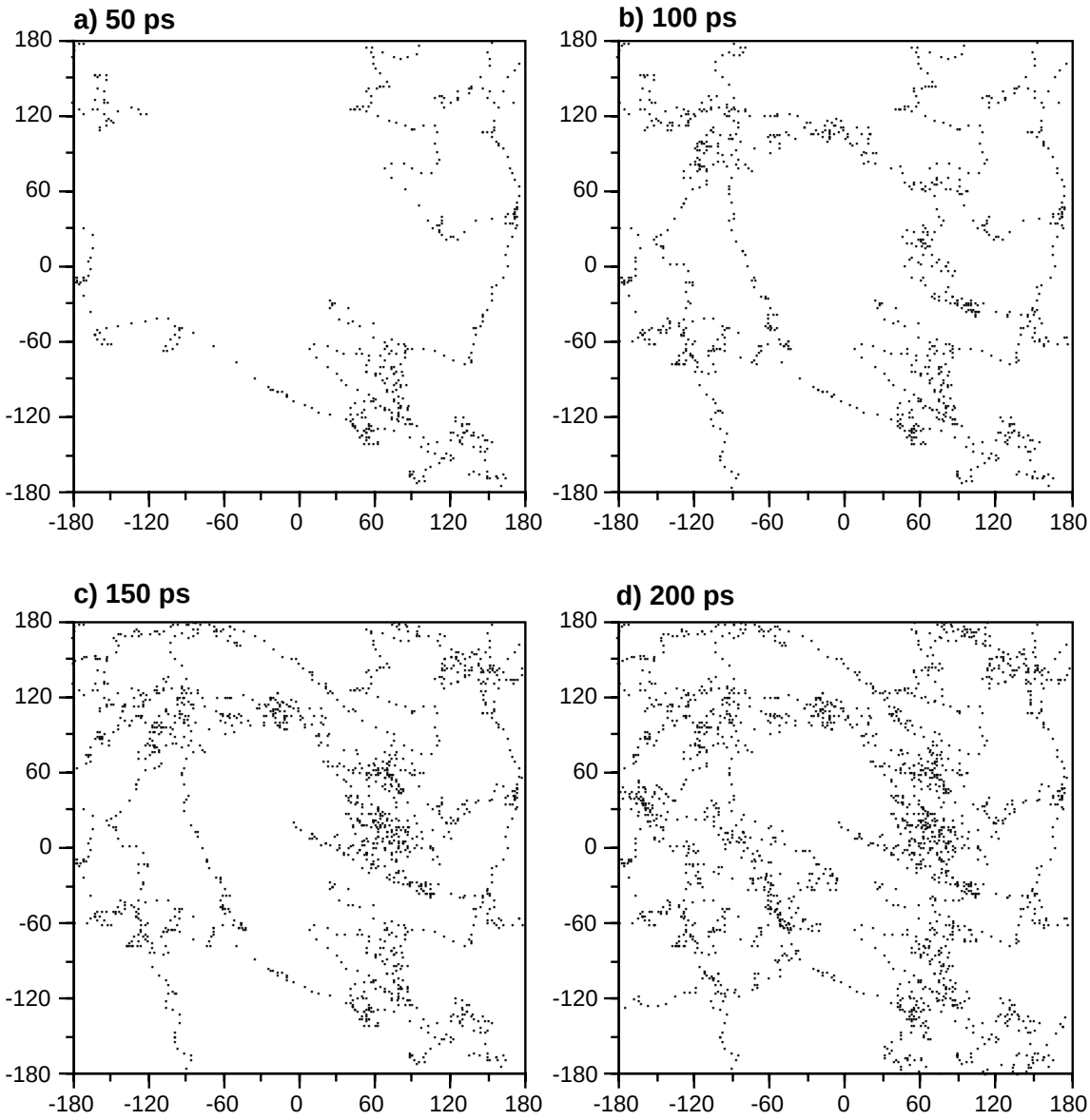

Fig. 4. ϕ–ψ trajectory of AAMA during the 200 ps MD calculation. The horizontal and vertical axes represent ϕ and ψ values in degrees, respectively.

In clustering the conformers of cyclodecane, we should note that the molecule is topologically symmetrical. Because CAMDAS performs the superposition and clustering with the absolute atomic numbers, the conformers of cyclodecane generated by CAMDAS would still include those which were close to each other. For example, when we superpose carbon 1 to 10 of one conformer on carbon 1 to 10 of another, respectively, and the resulting rmsd was greater than the threshold, CAMDAS would consider that those two were different. However, this pair might give a smaller rmsd value when we superpose carbon 1 to 10 of one on carbon 2 to 10 and 1 of another, respectively. Therefore, in the case of cyclodecane, though we think this is a special case, it is necessary to recluster the conformers resulting from CAMDAS. We did this reclustering by comparing the values of all of the possible pairs of the dihedral angles between the conformers. A threshold of 30° was adopted to distinguish two conformers. If the maximum deviation between the pairs of dihedrals was above the threshold, the conformers would be treated as different from each other.

Results

Conformational analysis of AAMA

We first evaluated the ϕ–ψ potential energy surface of AAMA by systematically rotating the related bonds. The obtained map for the energy surface is shown in Fig. 3. As indicated in Fig. 3, there were about four stable regions (local minima) on the energy surface generated with

TABLE 2
CONFORMERS OF AAMA FOUND BY CAMDAS

No.	Dihedral angles (°)		Relative energy (kcal/mol)[a]
	ϕ	ψ	
1	−97.3	26.7	0.00
2	62.4	30.3	0.46
3	−93.2	178.6	2.09
4	68.0	−178.2	3.16
5	65.7	−88.4	3.61
6	−77.3	119.5	3.76
7	155.4	−12.8	3.85

[a] Relative energy from conformer no. 1.

the force field used in CAMDAS. The lowest energy minimum was spread over the region of $\phi = -120°$ to $-60°$ and $\psi = -30°$ to 60°. This minimum includes C_7^{eq} conformation. The second minimum was on the region of $\phi =$ 30° to 90° and $\psi = 0°$ to 60°. This corresponds to the right-handed helix region observed in structures of proteins. In addition, two shallow minima were observed around $\psi = 180°$. We used this energy map as a reference in evaluating whether CAMDAS could find the representative conformers of AAMA.

Next we performed the MD and clustering calculation. In order to investigate whether the MD could search the conformational space adequately, we monitored the trajectories of ϕ and ψ dihedral angles during the MD calculation. The result is shown in Fig. 4. In this figure, the ϕ and ψ values of the conformers appearing in the MD were plotted. As indicated in the figure, the MD had already visited all four energy minima (Fig. 3) at 100 ps. At the end of the calculation (200 ps), the low-energy regions were almost searched by the MD. It is noteworthy that the high-energy regions (Fig. 3) have never been explored through the end of the MD. This indicates that CAMDAS could preferentially sample the energetically feasible conformers.

During the MD calculation, CAMDAS tested 2000 conformers appearing on the trajectory and roughly clustered them (see the Methods section). As a result, a total of 698 conformers were sampled during this stage. CAMDAS then minimized and reclustered these conformers. Finally, CAMDAS adopted seven from 2000 conformers as the representative conformers of AAMA. Table 2 shows these conformers with their ϕ, ψ values and the relative energy from the most stable conformer. Figure 5 indicates the positions of the adopted conformers on the potential energy surface. As shown in this figure, the four lowest conformers (conformers 1–4) represent the four energy minima on the energy surface, respectively. This result indicates that the CAMDAS could successfully find the low-energy conformations of AAMA. Three other

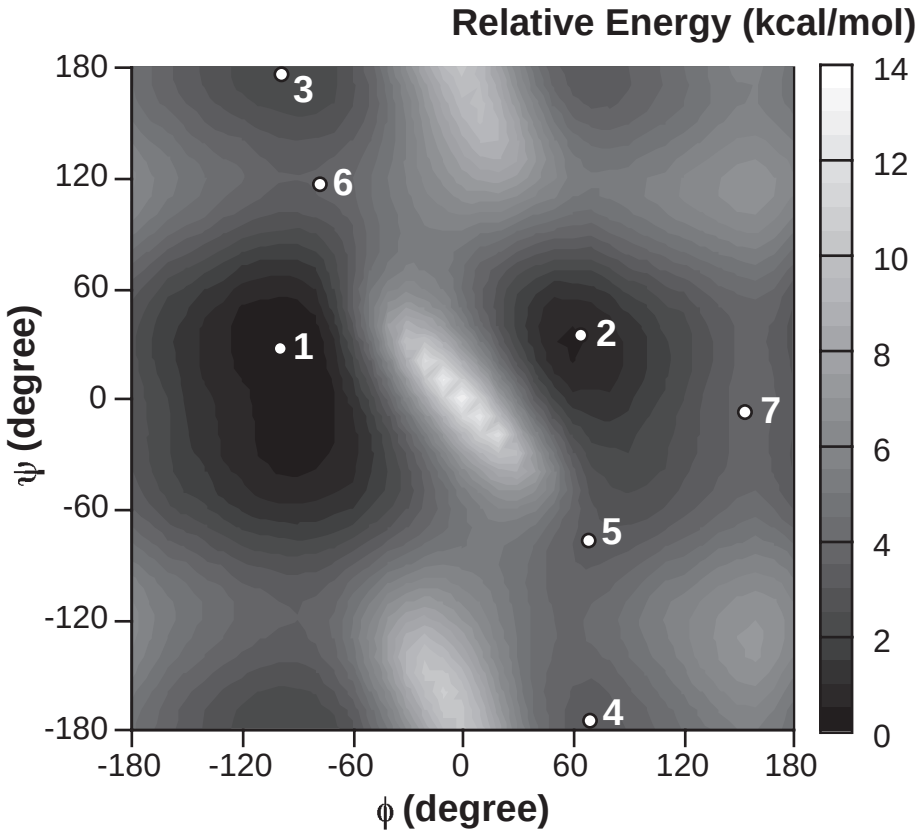

Fig. 5. The resulting conformers of AAMA obtained by CAMDAS. The potential surface of the molecule is also shown and the ϕ–ψ values of each conformer are plotted on this surface. The number on each conformer corresponds to that in Table 2.

TABLE 3
NUMBER OF CONFORMERS OF CYCLODECANE OBTAINED FROM THE TEST CALCULATION

Method of MD	Number of conformers		
	Examined during MD	CAMDAS	Reclustered
Normal	5000	221	21
Potential-scaled	5000	271	33

conformers were observed around these minima. This would be due to the relatively loose convergence criterion used in the minimization. If we use a stricter criterion, those conformers should converge on the minima on the potential surface of AAMA.

Conformational analysis of cyclodecane

Table 3 summarizes the number of conformers of cyclodecane generated by the normal and the potential-scaled calculations. In each calculation, a total of 5000 conformers appearing during MD were examined. Among them, the normal calculation extracted 221 conformers while the potential-scaled run selected 271 conformers. As described in the Methods section, these conformers extracted by CAMDAS would still contain similar ones. So we successively reclustered them in terms of the deviation of the dihedral angles. This resulted in 21 conformers for the normal calculation and 33 for the potential-scaled one. As a result, the potential-scaled technique could find more conformers for cyclodecane than the normal one did.

Tables 4 and 5 indicate the dihedral angles of the resulting conformers from the normal and potential-scaled calculations, respectively. Lipton and Still [7] have already studied the conformational behavior of cyclodecane with their modified systematic search method. They showed in their paper that there were 17 unique conformers of cyclodecane. So we compared our results with Lipton's conformers. This comparison is shown in Tables 4 and 5. Table 6 is a summary of the comparison. In Tables 4 and 5, the closest conformer of Lipton and Still is indicated for each of our conformers. As shown in the tables, the normal calculation could identify 13 of 17 Lipton's conformers. It also found eight conformational enantiomers of Lipton's conformers. The normal calculation failed to find Lipton's conformers nos. 7, 15, 16 and 17, though it could identify the conformational enantiomer of no. 7 (Tables 4 and 6). While the normal MD run missed some conformers, the potential-scaled MD successfully found all of 17 Lipton's conformers in the same calculation time as in the normal run (Tables 5 and 6). It could also find 14 conformational enantiomers. Interestingly, CAMDAS with the potential-scaled method identified two additional conformers that have not been reported in the literature (Table 5 and Fig. 6). These results clearly indicate that the potential-scaled technique could enhance the ability of searching conformational space during the MD.

TABLE 4
LIST OF CONFORMERS OBTAINED FROM THE NORMAL MD CALCULATION

No.	Dihedral angles (°)										Ref. conf.[a]
	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5	ϕ_6	ϕ_7	ϕ_8	ϕ_9	ϕ_{10}	
1	−151.3	55.3	65.5	−65.7	−55.2	151.4	−55.2	−65.6	65.5	55.4	1
2	52.2	−95.1	151.7	−64.8	−57.6	129.5	−57.3	−64.8	151.6	−95.2	2
3	−52.0	94.9	−151.8	64.8	57.5	−129.3	57.4	64.9	−151.8	95.0	2e
4	−138.5	61.5	76.5	−68.4	−68.3	76.6	61.3	−138.4	53.0	53.1	3e
5	138.4	−60.9	−76.8	67.9	68.8	−76.4	−61.5	138.5	−53.1	−53.1	3
6	−142.7	135.2	−83.9	60.5	−89.2	156.4	−60.9	−71.7	57.7	52.1	5
7	−145.1	145.5	−83.4	66.5	−83.4	145.3	−145.3	83.6	−66.7	83.2	4
8	−119.6	61.3	71.3	−140.8	108.9	−44.6	−60.3	153.1	−92.2	70.7	6
9	−108.5	131.5	−108.2	93.6	−54.0	−57.1	168.8	−57.5	−53.7	93.6	8
10	108.6	−131.2	107.7	−93.6	54.2	56.9	−169.0	57.4	53.9	−93.8	8e
11	−119.3	69.3	72.2	−149.9	48.5	44.2	−126.8	139.9	−87.0	81.0	7e
12	−125.1	54.7	64.8	−73.3	−72.0	72.8	72.5	−65.1	−54.2	124.9	10
13	151.6	−112.1	92.0	−50.2	−58.2	151.4	−112.1	92.0	−50.1	−58.4	9e
14	−151.5	112.6	−92.2	49.8	58.7	−151.5	112.5	−92.3	49.8	58.5	9
15	126.3	−69.5	91.4	−128.5	98.4	−107.0	67.2	65.7	−87.3	−64.2	12e
16	−126.8	69.9	−91.0	127.9	−99.2	107.7	−66.3	−66.5	87.3	64.4	12
17	−68.6	119.8	−87.7	120.3	−68.4	−68.6	119.9	−87.8	120.2	−68.3	11
18	−145.9	78.1	−79.7	118.0	−79.7	78.1	−145.9	53.9	25.7	53.9	14
19	145.8	−78.0	79.5	−118.0	79.9	−78.1	145.9	−53.9	−25.9	−53.6	14e
20	−123.3	87.9	−82.7	148.3	−96.5	36.4	−96.5	148.3	−82.7	87.8	13
21	123.1	−87.5	82.5	−148.7	94.7	−33.6	94.5	−148.7	82.5	−87.2	13e

[a] The conformer reported by Lipton and Still [7] corresponding to our conformer. Numbers are the same as those used in the literature. A suffix letter 'e' indicates a conformational enantiomer.

TABLE 5
LIST OF CONFORMERS OBTAINED FROM THE POTENTIAL-SCALED MD CALCULATION

No.	Dihedral angles (°)										Ref conf.[a]
	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5	ϕ_6	ϕ_7	ϕ_8	ϕ_9	ϕ_{10}	
1	−151.4	55.3	65.5	−65.5	−55.3	151.4	−55.2	−65.6	65.6	55.3	1
2	52.5	−95.5	151.5	−64.6	−57.5	129.7	−57.5	−64.6	151.5	−95.4	2
3	−52.1	95.3	−151.7	64.6	57.5	−129.6	57.6	64.6	−151.7	95.2	2e
4	68.0	−76.8	−61.0	138.4	−53.0	−53.2	138.5	−61.7	−76.3	68.7	3
5	−68.1	76.6	61.2	−138.4	53.1	53.1	−138.4	61.3	76.6	−68.5	3e
6	−142.7	135.0	−83.9	60.7	−89.2	156.3	−60.9	−71.7	57.8	52.2	5
7	142.8	−134.9	83.9	−60.9	89.5	−156.3	61.0	71.6	−58.0	−52.0	5e
8	−145.2	145.3	−83.3	66.4	−83.4	145.5	−145.2	83.3	−66.7	83.5	4
9	119.7	−61.3	−71.4	140.8	−109.0	44.7	60.3	−153.1	92.2	−70.7	6e
10	−119.5	61.2	71.5	−140.8	108.8	−44.6	−60.3	153.1	−92.1	70.6	6
11	108.4	−131.7	108.3	−93.5	53.9	57.2	−168.5	57.3	53.9	−93.6	8e
12	−108.4	131.8	−108.4	93.5	−53.7	−57.4	168.7	−57.2	−53.8	93.5	8
13	−138.0	85.3	−82.6	121.1	−68.6	−72.4	149.5	−48.4	−45.3	128.7	7
14	138.8	−86.6	81.9	−119.9	68.9	72.1	−149.9	48.6	44.9	−127.4	7e
15	−124.9	54.5	64.9	−72.7	−72.5	72.2	73.1	−64.8	−54.7	125.0	10
16	−151.5	112.1	−92.0	50.2	58.2	−151.5	112.2	−92.0	50.0	58.4	9
17	151.5	−112.1	91.9	−50.1	−58.3	151.6	−112.1	92.0	−50.2	−58.2	9e
18	−126.4	69.6	−91.1	128.3	−98.9	107.3	−66.6	−66.2	87.6	64.2	12
19	126.7	−70.0	91.1	−128.0	99.2	−107.6	66.4	66.5	−87.3	−64.4	12e
20	68.2	−120.6	88.0	−119.8	68.7	68.2	−120.6	88.1	−119.7	68.7	11e
21	−68.3	120.3	−88.3	120.3	−68.3	−68.3	120.2	−88.3	120.3	−68.3	11
22	145.8	−78.2	80.0	−118.0	79.6	−78.2	146.0	−53.8	−25.7	−53.9	14e
23	−145.8	78.1	−79.6	118.0	−79.7	78.0	−145.8	53.6	26.2	53.5	14
24	−123.3	87.8	−82.7	148.4	−96.6	36.6	−96.6	148.3	−82.7	87.9	13
25	123.1	−87.8	82.7	−148.3	96.2	−36.1	96.3	−148.4	82.8	−87.7	13e
26	−133.2	59.9	44.2	−97.3	139.7	−57.8	−72.3	37.2	66.5	−1.4	15
27	133.0	−60.0	−44.3	97.4	−139.7	57.8	72.3	−37.3	−66.7	1.9	15e
28	147.7	−122.2	43.9	41.9	−119.5	147.7	−122.1	43.6	42.1	−119.6	16e
29	−145.5	127.1	−47.4	−40.2	115.2	−145.5	127.2	−47.4	−40.2	115.3	16
30	109.2	−47.8	−76.2	21.3	77.0	21.9	−76.2	−48.1	109.3	−76.9	17
31	−109.3	48.1	76.3	−22.0	−76.8	−21.6	76.5	47.7	−109.2	77.0	17e
32	−156.2	110.2	−34.6	−48.2	128.9	−29.7	−96.8	29.1	37.1	41.4	*
33	−58.4	31.3	60.0	31.4	−58.7	−57.8	31.6	59.2	31.5	−58.1	*

[a] The conformer reported by Lipton and Still [7] closest to our conformer. Numbers are the same as those used in the literature. A suffix letter 'e' indicates a conformational enantiomer. An asterisk indicates the conformer that has not been reported by Lipton and Still.

Discussion

The purpose of CAMDAS is to find the representative conformers of a target molecule as easily as possible. We adopted molecular dynamics (MD) techniques as a driver for the conformational search. CAMDAS performs the integrated processes of sampling during MD, minimizing, and clustering in terms of the deviations of the atomic positions. In this way, CAMDAS automatically generated the representative conformers of the molecule from an initial structure.

First we examined whether CAMDAS could work correctly. In the conformational analysis of AAMA, CAMDAS finally extracted its seven low-energy conformers (Table 2). They contained several conformers that were not located at the energy minima, due to the loose convergence criterion in the minimization stage. Nevertheless, the four lowest energy conformers represented the four energy minima on the energy surface that was explored by the systematic search. This consistency with the systematic search results proved the validity of the strategy used in CAMDAS.

The detailed analysis of the ϕ–ψ trajectory of AAMA (Fig. 3) revealed a benefit of the MD technique. As shown

TABLE 6
SUMMARY OF THE COMPARISON OF THE RESULTS FOR CYCLODECANE WITH THE PREVIOUS SYSTEMATIC SEARCH RESULTS [7]

Method of MD	Missed conformers reported in Ref. 7[a]	Number of conformers that were not reported in Ref. 7
Normal	7[b], 15, 16, 17	0
Potential-scaled	None	2

[a] Numbers are the same as those used in Ref. 7.
[b] CAMDAS found its conformational enantiomer.

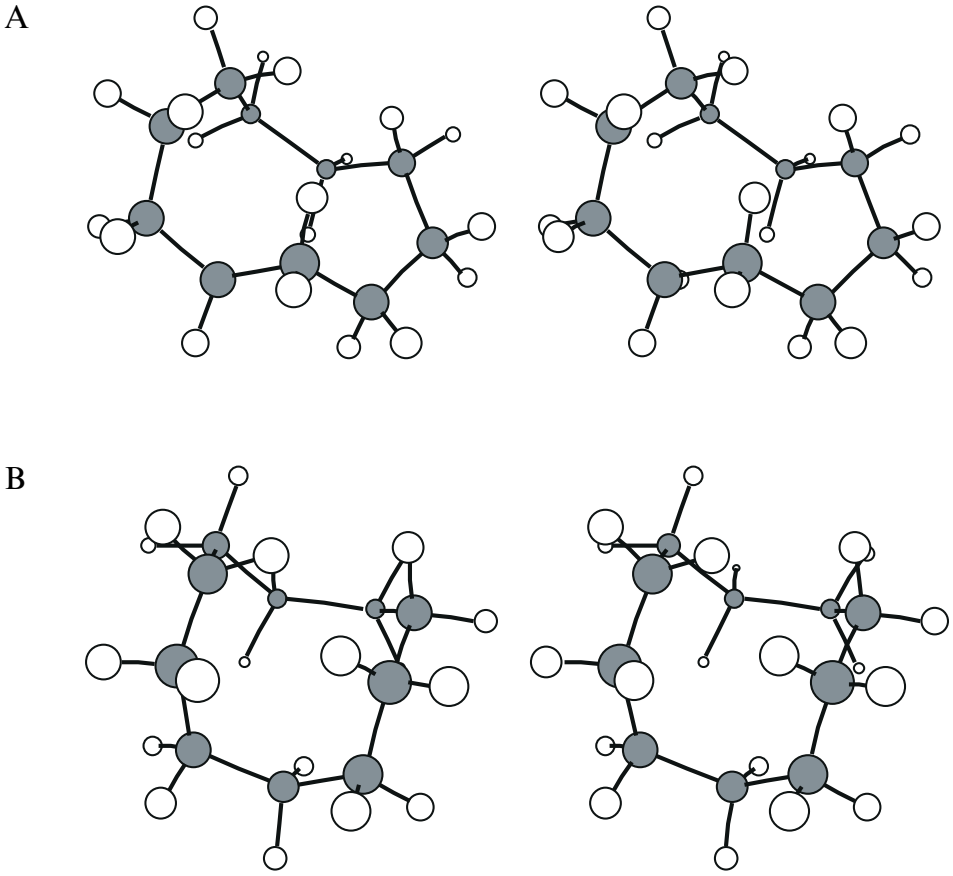

Fig. 6. Stereoviews of the newly found conformers of cyclodecane: (A) no. 32 in Table 5; (B) no. 33 in Table 5.

in the figure, the low-energy regions were preferentially searched while the high-energy regions were rarely visited. This is an advantage over the other well-known techniques such as the systematic search or Monte Carlo methods. Because these methods move the molecule uniformly or randomly, it is difficult to suppress the occurrence of high-energy conformers. This would decrease the efficiency of the methods. In our case of AAMA, the systematic search method might be more efficient than the MD one because the molecule has only two degrees of freedom (ϕ and ψ torsion angles). However, if the target molecule becomes more complex and has a greater number of degrees of freedom, MD would be more efficient than the systematic search.

The second example of cyclodecane was performed to examine the utilities of CAMDAS in the conformational analysis of a cyclic system. As indicated in Tables 4–6, CAMDAS could extract most of the known conformers of cyclodecane that have been reported by Lipton and Still [7] with the systematic search. CAMDAS also found several conformational enantiomers of Lipton's conformers. Although Lipton and Still intended to avoid the generation of enantiomeric conformations in their work, we think that the enantiomeric conformers are also important in the drug-design process because it is possible that an enantiomeric conformer might be a biologically important one. CAMDAS could identify these conformers by examining only 5000 conformers sampled from the MD calculation. If one tried the systematic search by rotating each of 10 bonds in cyclodecane, one must evaluate a huge number of conformers. Moreover, the systematic search method must be modified with some complex techniques such as bond-breaking in order to handle cyclic compounds properly. In contrast, the only parameters important for CAMDAS are the atomic positions and forces. It has no concern with the topology of the molecule. CAMDAS just calculates the movement of the atoms by Newton's law, samples conformers, and clusters them by the difference in their atomic positions.

We have implemented an accelerating method, the potential-scaled technique, in CAMDAS and have evaluated its usefulness. In this method, the angle and torsional terms of the potential were reduced to one-half their original values. This is intended to make the conformational transitions easier by lowering the potential energy barriers. In the example of cyclodecane (Tables 3 and 5), the potential-scaled method could identify all of the known conformers while the normal MD missed some of them. In addition, the method detected two additional conformers that were not reported by Lipton and Still [7]. These

two conformers have rather high energies and include some hydrogen atoms that are close to each other. Lipton and Still might have missed these conformers because of the close van der Waals contacts in the conformers. Anyway, our results indicate that the conformational space was searched more widely with the potential-scaled method. One may wonder if the appearance of many conformers in the potential-scaled method was due to changing the potential function from the original. We think this is not true. We have previously proven that the reduction of the angle and torsional terms by half did not alter the conformational behavior of the molecule [8]. In addition, the conformers sampled by CAMDAS are finally minimized, and the potential used in the minimization is not reduced. Therefore, even if the conformational properties are slightly modified in the MD, the following minimization process should adjust the artificial conformations.

Conclusions

We have developed an automated conformational analysis program, CAMDAS, and have proven its validity through several examples. Using MD techniques, CAMDAS could extract the representative conformers of the target molecules easily and efficiently. In addition, CAMDAS can handle any type of molecule such as a cyclic compound. CAMDAS presents a convenient way to find most of the energetically possible conformers of a molecule, which is needed especially in the early stage of drug design.

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