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High-Temperature Simulation of Dynamics of Cyclohexane

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The dynamic behaviors of the cyclohexane molecule have been simulated by normal- and high-temperature molecular dynamics calculation. At 1000 K, all of the well-known conversion processes and equilibria between conformers (chair form inversion, boat-chair conversion and pseudorotation) were fully reproduced in the trajectories within a practical simulation time, 50 ps, whereas at normal temperature (300 K), these conversions scarcely occurred even during an extraordinarily long simulation time, 100 ns. The calculated structures and relative stabilities of conformers agreed well with the experimental data. These satisfactory results with this well-studied molecule imply applicability of unrealistically high-temperature simulation to the prediction of dynamic behaviors of other molecules.

Keywords cyclohexane; molecular dynamics; conformational conversion; pseudorotation

Molecules continuously undergo structural fluctuations. Usually, in a conformationally flexible molecule, only the most stable conformation or an average of multiple conformations in rapid equilibrium is observed experimentally, although individual conformations can be identified in rare cases. Moreover, structures determined experimentally are the time-averaged and position-averaged ones, because experiments must be performed on a mass of molecules and a certain period of time is required to measure physical quantities. For example, signals in NMR spectra reflect all kinds of structural fluctuations. Even the structures derived from X-ray analyses are the averaged ones with respect to the internal motions and disorders allowed in the crystal.1) Thus, it is difficult to discuss the actual structures, to say nothing of the actual behaviors, only on the basis of experimental studies, although they are the essential basis of structural chemistry.

Based on molecular modeling and computer techniques, many simulation studies have been conducted to interpret experimental observations. By molecular mechanics (MM) calculation,2) the energetically optimized structure and minimized energy value can be obtained without experiments, using empirical energy functions and force field. The obtained structure is not an averaged one. The method can treat the conformational diversity of molecules to some extent by utilizing various starting structures. But it is not sufficient for analyzing dynamic behaviors of molecules because the results are simply a set of separate structures whose internal motions are quenched.

Recently, it has become possible by molecular dynamics (MD) calculations3) to understand various molecular phenomena that cannot be explained from the static viewpoint. MD calculation can simulate the actual motion of a molecule, based on the same force field as in MM calculation and Newton's equations of motion assuming each atom to be a particle. Successive integrations of these equations lead to the trajectory of each atom over time, which is given as a list of atomic positions and velocities. The analysis of the trajectory over a certain period of time reveals structural and energetic details of the continuous internal motion of the molecule. Unlike the MM calculations, MD calculations do not converge to energy minima. The structures fluctuate infinitely in geometrical and conformational space, overriding energy barriers. By tracing the motions of a molecule along the time course, it should be possible to look more deeply at the characteristic structures and motions inherent in an individual molecule, than it would be by treating a mass of the molecules.

However, MD calculation cannot always reproduce all conformational behaviors within a practical simulation time when it is performed at normal temperature. It often occurs that stable conformers and equilibria between them that are experimentally observed cannot be reproduced by the calculation, depending on the input starting structure and on the height of energy barriers between stable states. This situation is not due to defects in the theory or parameters of the MD calculation, but is ascribed to the short simulation time (100—1000 ps) compared to the actual time-scale of motions in molecules. Since the transitional probability of overriding a high energy barrier increases with the temperature, unrealistically high temperature is effective for searching a conformational space without preconception. Therefore, high-temperature MD (HTMD) calculation has often been used in combination with MM calculation for the purpose of searching possible conformers and predicting structures and relative stabilities of the conformers.4)

However, as regards dynamic behaviors of molecules, not enough is known about whether HTMD calculation can satisfactorily reproduce actual motions of molecules that are observed with various experimental methods or proposed speculatively. So, in order to assess the validity of the method, we have performed MD calculations at normal and high temperatures on the cyclohexane molecule, whose conformations and dynamic behaviors have been well studied by various experimental methods such as infrared spectroscopy,5) NMR spectroscopy,6) and EPR spectroscopy.7)

Together with its conformations, internal motions of cyclohexane have been attracting our interest for a long time because of the high molecular symmetry and high conformational mobility.8) The existence of several typical conformers of the cyclohexane ring and their relative stabilities were estimated from conformational studies on the molecule and its derivatives with various substituent groups.[9)] Processes of conversion among the conformers were also well studied, by searching for the energetically most favorable pathways.[10)] Furthermore, as a characteristic motion common to highly symmetric and cyclic molecules such as cycloalkanes, a pseudorotation is supposed to exist between two conformers that are geometrically and energetically close to each other.[11)] All these results were not obtained only from experimental observations, but rather from speculations and calculations on various models based on the observations.

In this paper, the reproducibility of the equilibrium among conformers of cyclohexane is examined first by MD simulations at various temperatures. Then, the validity of high-temperature MD calculation for the study of dynamic behaviors is confirmed by analyzing in detail the processes of interconversion among conformers, and comparing the results with the reported experimental results and speculations.

Experimental

The AMBER program version 3.0[12)] was used for the MD calculation, energy minimization, and following analyses. Hydrogen atoms were treated explicitly in all the calculations. The atom-types of "CT" and "HC" in the force field parameters of the AMBER program[13)] were used for carbon and hydrogen atoms, respectively. The formal charges on the carbon and hydrogen atoms used were -0.10 and $+0.05$, respectively. The value of dielectric constant was assumed to be the same as the distance between atoms.

Starting structure was roughly modeled using standard bond lengths and bond angles, and was energy-minimized. After a gradual increase of the temperature to the specified value, 5.0 ps (1 ps $= 10^{-12}$ s) dynamics calculations were performed for the equilibration of the whole system. After that, molecular dynamics trajectories were calculated using a time step of 0.001 or 0.0005 ps. Frame data of the trajectories, that are composed of atomic positions, velocities and energies, were stored in files at regular intervals.

In this study, we performed two modes of the MD calculations and subsequent analyses, that is, preliminary and accurate modes. In the preliminary mode, trajectories were stored every 50 steps of MD calculation, in order to check roughly the occurrence of the target conversion. The starting time and conditions for the accurate-mode calculations were chosen based on the result of the preliminary-mode calculation. In the accurate mode, calculations were restarted in limited ranges of time course and the frame data were stored at every MD step. Several temperature settings were tested in the preliminary phase, and a suitable one was selected at which a sufficient equilibration was completed within a certain period of time. The rescaling of velocities was not performed within a run.

As the structures in the trajectory were chaotic due to the atomic motions, they were subjected to energy minimization to give the quenched structures in order to compare their stabilities or to classify them into the typical conformers. Energy minimization was done by the first 10 cycles of the steepest-descent method followed by the conjugate gradient method until convergence was obtained. We have modified the AMBER minimization program so that all the frame structures are successively subjected to the energy minimization. The energy-minimized structures were classified into typical conformers by the template fitting method. The structures were superposed on a group of template structures one after another, by a least-squares calculation. Similarity between the superposed two structures was judged on the basis of the value of the root mean square deviation of all atoms. A structure with the value of less than or equal to 0.005 nm was judged to be almost the same as the template structure and classified into the corresponding typical conformer. If the values with all the template structures were more than 0.005 nm, the structure was added as a new member to the group of template structures. Programs for the template fitting, analysis of energies and structures, and for visualization have been developed in our laboratory. All calculations were performed on IRIS 4D/220 and 4D/420 workstations.

Results and Discussion

Five conformers, chair (C) form, skew-boat (SB) form, boat (B) form, half-chair (HC) form and sofa (S) form, are known as typical conformers of the saturated six-membered ring,[14)] although the chair form is the only conformer which is experimentally observed for unsubstituted cyclohexane. For each typical conformer, there can be a number of equivalent structures, when we discriminate the six carbon atoms and the two sides of the ring plane. There are two equivalent structures for the chair form, six for the skew-boat and boat forms, and twelve for the half-chair and sofa forms. For the purpose of conventional (static) conformational analyses, it is sufficient to search only typical stable conformers. But from the viewpoint of dynamic behavior of molecules, energetic and structural analyses of the interconversion processes are essential while taking into account unstable transition states and equivalent conformers.

First, the occurrence of interconversion between chair form and boat (or skew-boat) form was examined at various temperatures and with various simulation periods. At normal temperature (300 K), the interconversion never occurred within moderate simulation periods (100—1000 ps). Even in an extraordinarily long simulation of 100 ns, the interconversion occurred only 180 times. At 500 K, the interconversion occurred 372 times within a simulation of 50 ns. At 1000 K, the interconversion occurred quite frequently (29 times within 50 ps).

The time course of potential energy obtained from the simulation for 50 ps at 1000 K and that from the corresponding energy-minimized structures are shown in Fig. 1. As can be seen from Fig. 1a, the time course of energy in the trajectory does not provide any useful information due to the large fluctuation of the energy value, whereas that of the energy-minimized structures clearly shows the appearance of conformers with four different energy values, as shown in Fig. 1b. The appearance of all five typical conformers was confirmed by the template fitting procedure applied to the energy-minimized structures. Due to the small energy difference, the half-chair and the sofa form could not be distinguished by the energy values. By comparing the torsion angles with the known values, the typical conformers could be discriminated easily. The relative energy values of 5.7, 6.9, 11.3 and 11.3 kcal/mol with respect to the chair form are in good agreement with the known values listed in Table I.

Chair-Form Inversion Process In the ^{1}H-NMR spectra of undecadeuterated cyclohexane, two singlet signals observed at $-86\,^{\circ}$C fused into one singlet signal at $-67\,^{\circ}$C.[6a)] This result indicated that an equilibrium exists between two chair forms, where the hydrogen atom at an equatorial position in one chair form takes an axial position in another chair form. This conversion, so-called "chair form inversion," was supposed not to occur in a single step but to occur *via* more than one typical conformer as a low energy pathway from studies using molecular models. From the temperature of the signal fusion, the energy barrier for chair form inversion was estimated to be 10.3 kcal/mol. Based on these results, it has been accepted that the chair form inversion proceeds

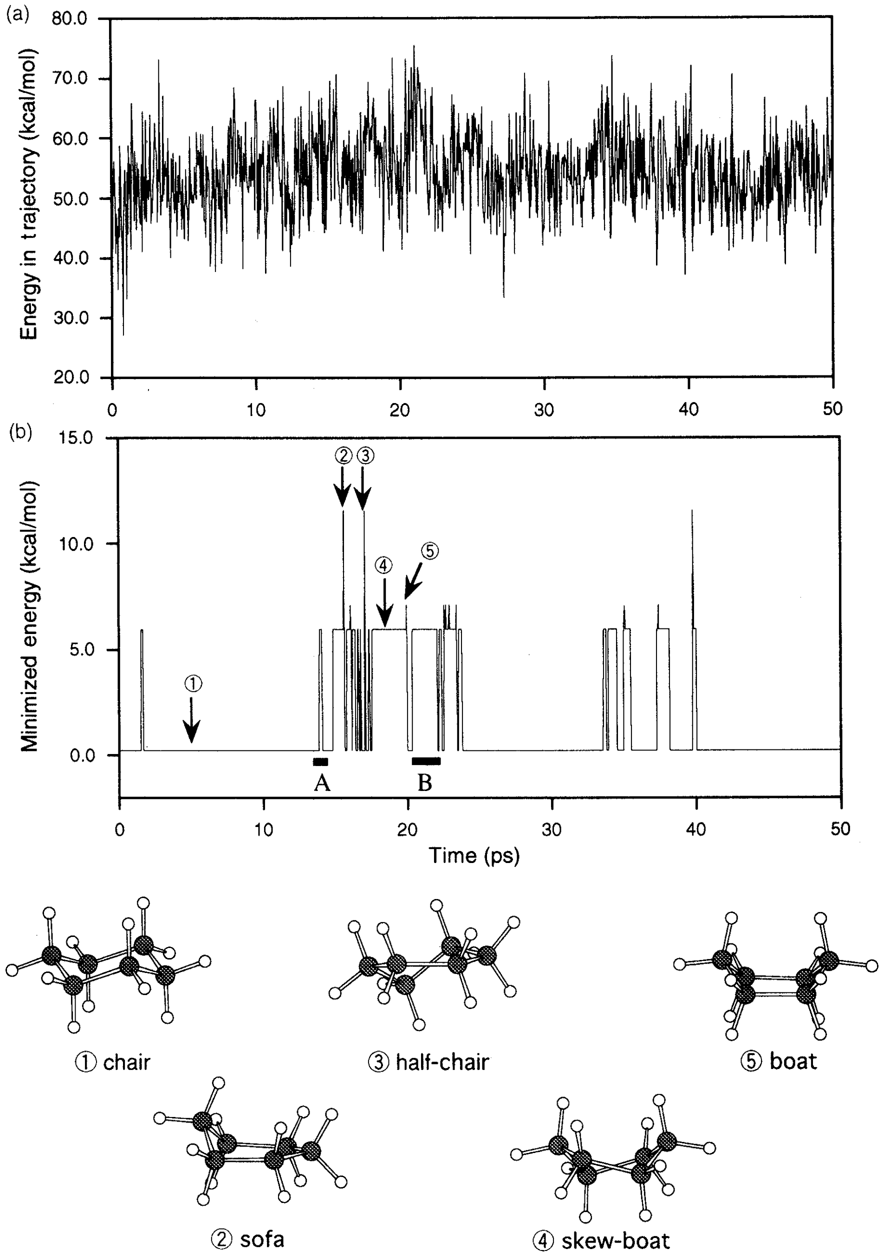

Fig. 1. A Preliminary Analysis by MD Calculation for Cyclohexane at 1000 K

(a) Time course for potential energy in the MD trajectory. (b) Time course for minimized potential energy together with snapshot structures at the positions ①—⑤ indicated by arrows.

Table I. A List of Relative Stabilities and Torsion Angles of Typical Conformers of Cyclohexane Obtained from This Work

Conformation	Energy in kcal/mol	Torsion angles in degrees					
Chair	0.00	+58.4	−58.4	+58.4	−58.4	+58.4	−58.4
	(0.0)	(+54)	(−54)	(+54)	(−54)	(+54)	(−54)
Skew-boat	5.73	+31.7	−66.2	+32.0	+31.5	−66.0	+31.8
	(5.5)	(+30)	(−63)	(+30)	(+30)	(−63)	(+30)
Boat	6.87	+52.5	+2.6	−55.3	+52.3	+2.8	−55.5
	(6.5)	(+52)	(0)	(−52)	(+52)	(0)	(−52)
Half-chair	11.29	+12.3	+8.4	−50.6	+78.7	−52.5	+10.3
	(11.0)	(+12)	(+8)	(−47)	(+67)	(−47)	(+8)
Sofa	11.33	+9.0	+25.1	−64.0	+69.3	−35.0	−3.8
	(11.2)	(+7)	(+28)	(−62)	(+62)	(−28)	(−7)

Values in parentheses are the corresponding energy and torsion angles taken from ref. 8*b*.

via unstable transition state conformers.

In order to examine the process of the chair form inversion, accurate calculations were performed at 1000 K within a number of time-ranges, where the inversion from one chair form to another chair form occurred in the preliminary calculation. It was shown that at least one boat family conformer (skew-boat form and boat form) invariably exists between the two chair forms. It was also shown that the unstable conformers, half-chair form and/or sofa form, invariably appeared between chair form and boat form family, although they could be found only occasionally in the preliminary analyses. Thus, the phenomenon of chair form inversion can be replaced by the conversion from the chair form to the boat form family and the reverse conversion. In the reverse step, the probability of transition to the inverse chair form is supposed to be one half. As an example of the chair form inversion process, Fig. 2 shows the result of accurate calculation for 0.25 ps in the range A indicated in Fig. 1b. The time course of the minimized energy and the corresponding structures of the molecule (Fig. 2a) show the inversion process through a sequence of typical conformers. The time course of the six torsional angles along the ring carbons represents a concerted but asymmetrical process of the inversion, as shown in Fig. 2b. A chair form has reached another chair form, by way of a half-chair form, a skew-boat form and another half-chair form. This was the shortest pathway of the chair form inversion. Because a rapid interconversion occurs between skew-boat form and boat form due to pseudorotation, which will be described later, the chair form inversion usually occurs by way of more than one

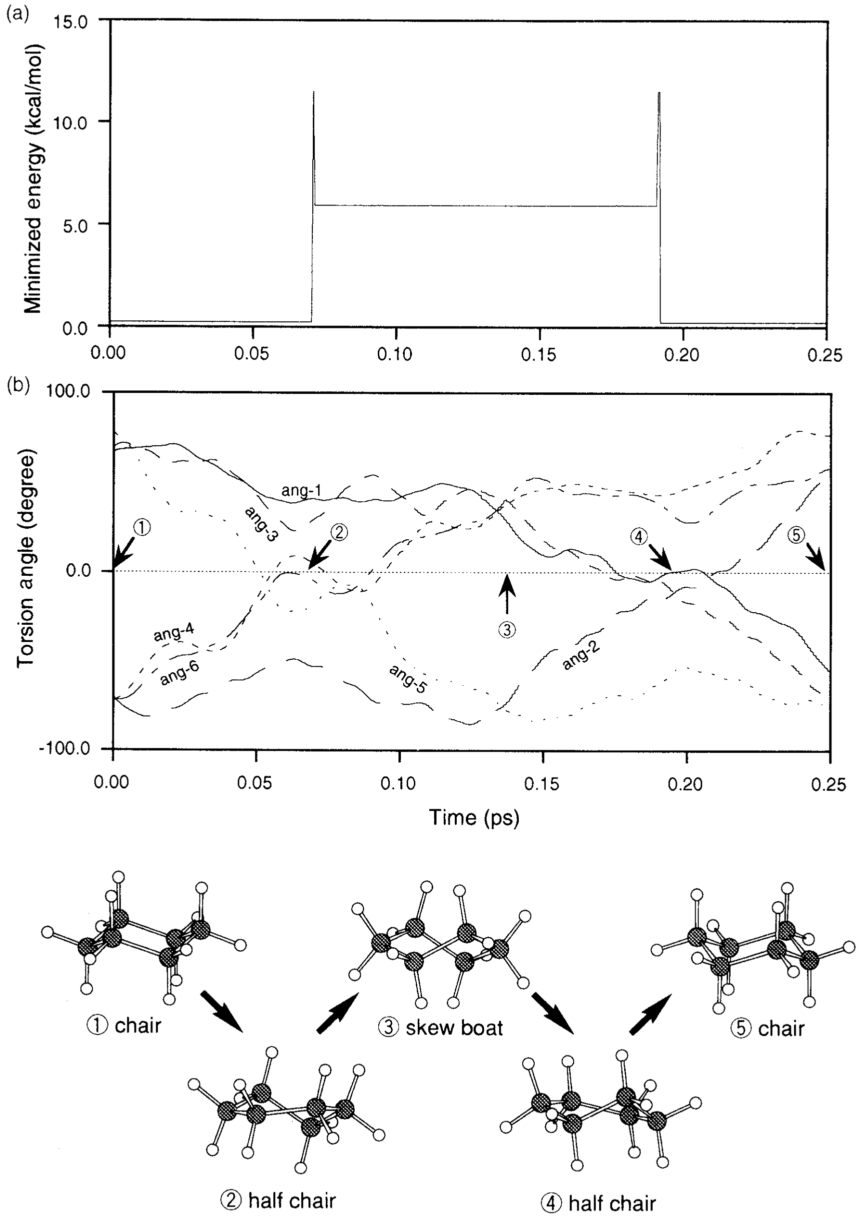

Fig. 2. Process of the Chair Form Inversion in Cyclohexane, from the Accurate MD Calculation at 1000 K in the Time Range A Indicated in Fig. 1b

(a) Time course for minimized potential energy. (b) Time course for six torsion angles in the trajectory together with snapshot structures at the positions ①—⑤ indicated by arrows. Ang1—ang6 designate successive torsion angles in the six-membered ring.

boat form family conformer between two unstable transition-state conformers. Accordingly, there can be innumerable pathways for the chair form inversion.

Transition State As for the exact structure of the transition state conformer of chair-form inversion, it has long been in question whether it is the sofa form or the half-chair form. Molecular mechanics calculations on cyclohexane have been carried out by many different groups.$^{10)}$ Quantitative comparisons of the stabilities of both conformers were made by using various force fields. Models of the inversion process were proposed taking into account the structural continuity between the equivalent structures of the five typical conformers.

In our calculations, all four pathways to the boat family, which are *via* sofa to skew-boat, *via* sofa to boat, *via* half-chair to skew-boat and *via* half-chair to boat, were proved to be possible, starting from a chair form structure. It was also proved that the conversions proceed without conservation of the molecular symmetry. From the accurate analyses, it was also shown that a pseudorotation can exist between half-chair form and sofa form, although it did not appear often due to the instability of these forms. The half-chair, sofa and boat forms are not energy-minimum states, but are saddle-point states, which cannot be picked up by some energy minimization algorithms. They could be detected, as well as the local-minimum states, namely chair and skew-boat forms, by the minimization method adopted in the AMBER program. It is noteworthy that such unstable transition-state conformers could be trapped with exact structures and with exact measures of relative stabilities in our calculation.

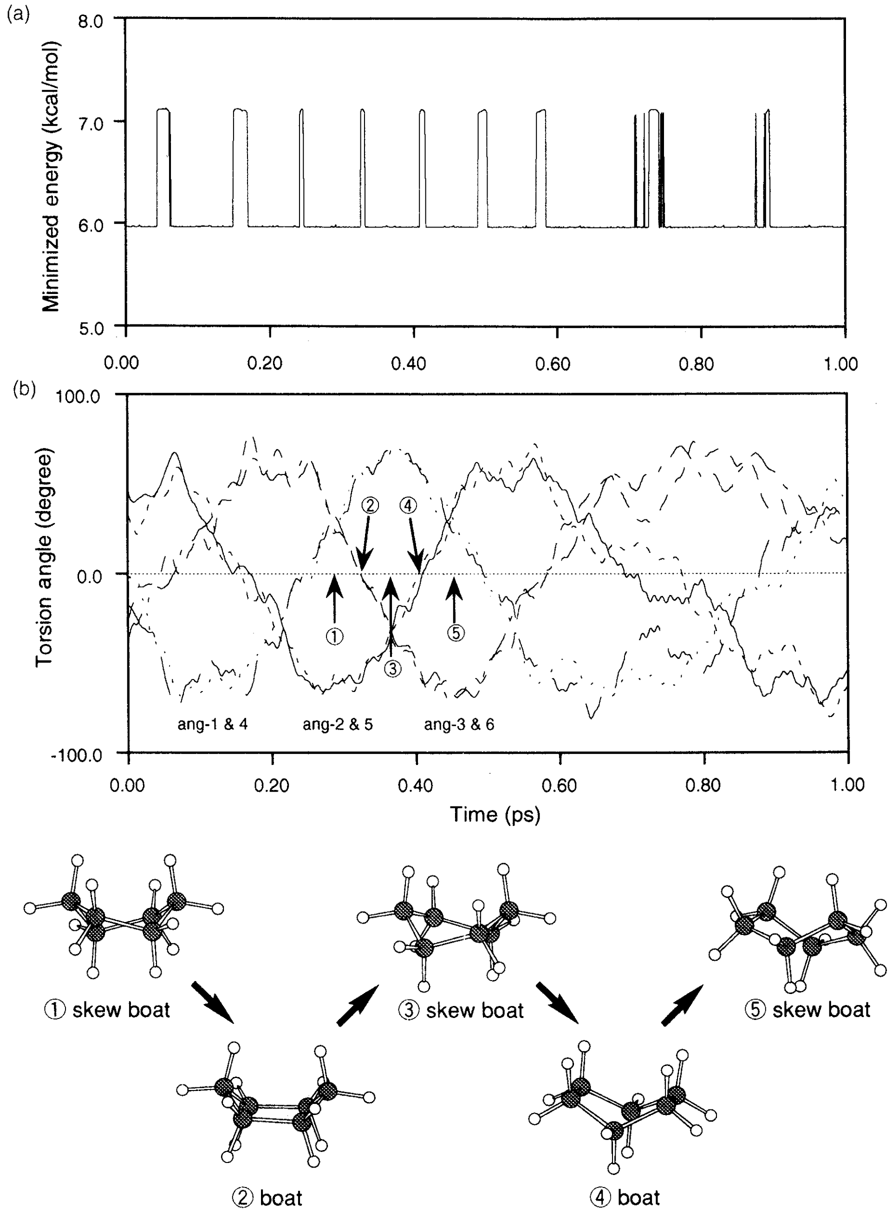

Fig. 3. Process of the Pseudorotation between Skew-Boat Form and Boat Form in Cyclohexane, from the Accurate MD Calculation at 1000 K in the Time Range B Indicated in Fig. 1b.

(a) Time course for minimized potential energy. (b) Time course for torsion angles in the trajectory together with snapshot structures at the positions ①—⑤ indicated by arrows. Ang1—ang6 designate successive torsion angles in the six-membered ring.

Pseudorotation In small, cyclic and highly symmetric molecules, there are many equivalent structures and consequently there are many equivalent motions among them. The motion from one conformer to another conformer is likely to propagate successively along the ring, rather than being confined to a simple forward-and-backward motion between the two structures. Such consecutive circular motions are called pseudorotation. In cyclohexane, pseudorotation is known to exist between the skew-boat form and the boat form.[15]

The details of the pseudorotation were made clear by the accurate analysis of the time range where a rather long lifetime of the boat family conformation was observed in the preliminary analysis. The accurate calculation at 1000 K restarted in the range B (indicated in Fig. 1b) gave a typical example of pseudorotation. As shown in Fig. 3a, the time course of the minimized energy clearly shows systematic and alternate appearances of two conformers with the energy values of 6.0 and 7.1 kcal/mol, respectively. The time course of the six torsion angles in the ring shows the alternate appearance of six equivalent structures for typical conformers in a definite sequence, as shown in Fig. 3b. Each pair of torsion angles facing across the ring changes together and the three pairs of the angles change regularly to form a net-mesh pattern. This means that a rapid circulation of highly systematic and symmetric motions occurs in the six-membered ring. At 1000 K, it takes 0.5 ps for one cycle. It turned out that the cycle time increases as the temperature lowers. But, the result cannot be confirmed quantitatively, because no experimental value is available.

Overall Dynamic Behaviors Macroscopically, the internal motion of the cyclohexane molecule is composed of conversion between chair form and boat form family *via* transition state conformers, and pseudorotation in the boat form family conformers. Since a rapid equilibrium exists between two equivalent structures of the chair structure, unstable conformers that are hidden behind the exclusively stable chair form also play important roles in the actual behavior of the molecules. Although the dynamic behavior of molecules should be understood in terms of the MD structures in trajectory, it seems to be convenient to interpret the behaviors as sequences of equivalent structures of typical conformers. The MD calculation and subsequent analyses gave us the sequences of equivalent structures in the conversion processes without any assumption on stability and symmetry. The equivalent structures involved in the pseudorotation do not exist independently as static entities, but result probabilistically from the dynamic motions of the molecule. The dynamic aspects of the cyclohexane molecule shown in Fig. 4 were derived from the calculation in terms of the equivalent structures to the typical conformers.

Validity of High-Temperature Simulation It was shown that high-temperature (1000 K) MD calculation was very powerful for covering all typical conformers and all important processes of conformational conversions within a practically allowed simulation time, although it seems difficult to determine the most appropriate temperature of

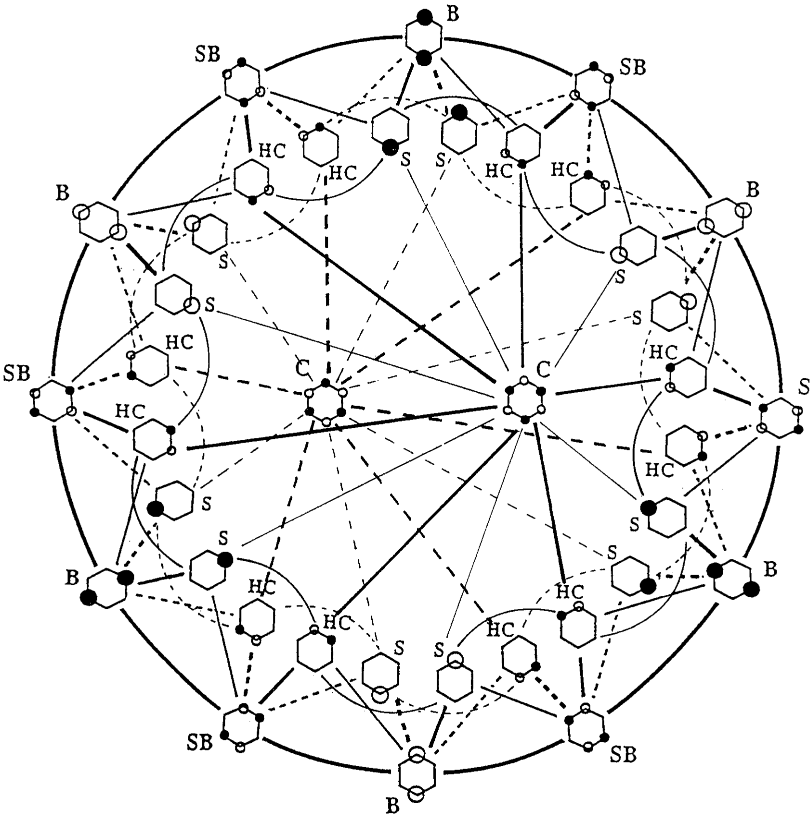

Fig. 4. A Schematic Representation of Conformational Interconversion between Equivalent Structures of Five Typical Conformers in Cyclohexane

Lines connecting pairs of conformers indicate possible pathways of direct interconversion. A circle on an atom indicates deviation of the atom from the mean plane of the ring (open circle: above the plane, filled circle: below the plane). The radius of the circle indicates the amount of the deviation.

simulation. High potential energy of the whole molecule is not a sufficient condition but a necessary condition for the conformational changes to occur. Some concerted motions of atoms seem to be important for the changes.

The high temperature increases the existence ratios of high energy states. Therefore, simulation at an abnormally high temperature might predict false pathways besides the actual one, going through other transition states with higher energies. Moreover, high-temperature simulation brings about unnecessary fluctuations in the time course of torsion angles. In order to check the extent of such effects, we compared the time course obtained from a simulation at 300 K, in which a chair-form inversion occurred, with that from a simulation at 1000 K. It was proved that the inversion process is well reproduced by the simulation at 1000 K as compared to that at 300 K.

It is ideal to perform the MD calculation at normal temperature in order to simulate the actual behavior of molecules. But, at normal temperature (300 K), it would take 10^5—10^7 ps of simulation time to reproduce the full equilibrium of conformers even in the cyclohexane molecule. This seems to be impossible for the time being, due to the current limitations of computer power. For example, the 100 ns (10^5 ps) simulation of cyclohexane took about 8 d by using a high-performance workstation (CPU R4000, 100 MHz). Therefore, we have to accelerate the conversion by raising the temperature of the simulation, in order to reproduce actually occurring motions within a simulation time which is practically possible. The frequencies of the appearance of various structures vary depending on the temperature according to Boltzman's law of distribution, and they are affected by the entropic contribution to the free energy. Accordingly, transitions which scarcely occur at normal temperature are found more often and the period of pseudorotation becomes shorter in the high temperature MD calculation. Since the fundamental processes of conformational conversion as well as the structures and energetics of this well-studied molecule could be fully reproduced and interpreted as shown in this paper, we can conclude that the high temperature MD simulation is a powerful tool for elucidating the actual motions of molecules at room temperature.

The calculated energy values agreed fairly well with the observed ones. The force field parameters of the AMBER program proved to be suitable for small cyclic hydrocarbons. As the errors resulting from inaccuracy of the force field accumulate during extended MD calculation, the results of MD calculation are more sensitive to force-field accuracy than those of MM calculation. And, the larger the molecule is, the more the error accumulates. In order to examine the reliability of the method, algorithm, parameters and procedures, it is necessary to carry out calculations on small and well-studied molecules, which have two or more stable structures and energies that are known experimentally. There is no means to verify the motions and behaviors in a system for which only one stable structure is known, even if the structure is determined by X-ray crystallography.

Even in the case of phenomena which can be superficially interpreted in terms of static aspects, information on dynamic properties might provide a better understanding. Experimental techniques for investigating the dynamic aspects of molecules are far from satisfactory at present. In this study, we have shown that MD calculation can complement experiments and can provide new insights which could not be obtained by experiments, as well as being able to reproduce the experimental facts. The method will be useful for predicting dynamic conformational properties of unknown or poorly studied compounds.

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