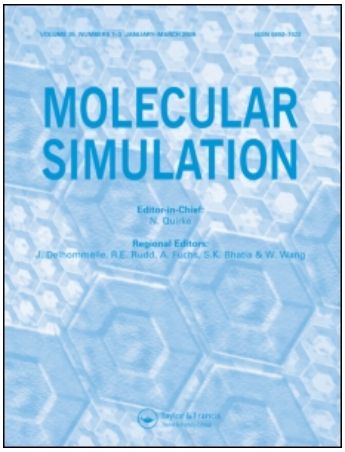

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Targeted Molecular Dynamics Simulation of Conformational Change-Application to the T ↔ R Transition in Insulin

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TARGETED MOLECULAR DYNAMICS SIMULATION OF CONFORMATIONAL CHANGE - APPLICATION TO THE T ↔R TRANSITION IN INSULIN

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A novel method to calculate transition pathways between two known protein conformations is presented. It is based on a molecular dynamics simulation starting from one conformational state as initial structure and using the other for a directing constraint. The method is exemplified with the T ↔ R transition of insulin. The most striking difference between these conformational states is that in T the 8 N-terminal residues of the B chain are arranged as an extended strand whereas in R they are forming a helix. Both the transition from T to R and from R to T were simulated. The method proves capable of finding a continuous pathway for each direction which are moderately different. The refolding processes are illustrated by a series of transient structures and pairs of Φ, Ψ angles selected from the time course of the simutations. In the T → R direction the helix is formed in the tast third of the transition, while in the R → T direction it is preserved during more than half of the simutation period. The results are discussed in comparison with those of an atternative method recently apptied to the T → R transition of insulin which is based on targeted energy minimisation.

KEY WORDS: Conformational transition, MD simulation, Insulin

INTRODUCTION

Molecular dynamics (MD) simulations have proved to be extremely helpful in the analysis of the changes leading to different conformational states which a protein may adopt in different environments [1-4]. The changes accompanying integration of an isolated molecule in a quaternary structure or a crystal lattice are normally very small. Much larger changes can be induced and analysed by ordinary MD simulation at deliberately elevated temperatures [5-7]. Extensive changes between two defined structural states, however, have no chance to be treated by ordinary MD simulation within present limits of computation [8]. Different approaches have

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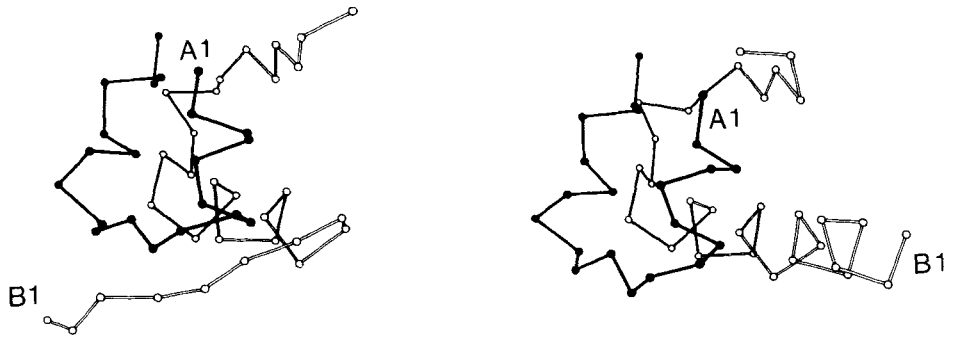

Figure 1 Ball-and-stick representation of the C_α-structures of T (left) and R (right). The N-termini of the A chain (bold) and B chain are marked.

been established to overcome this problem which induce the desired motion by by the introduction of a certain bias in the force field [9–11].

In a previous paper [12] we presented a method to calculate the T ↔ R extensive conformational change using "targeted energy minimisation" (TEM). In the present communication the problem is tackled by a novel method apostrophised "targeted molecular dynamics" (TMD). One limiting structure of a transition serves as the starting geometry for the simulation, the other is used to provide a directing constraint. Pathways can be calculated even if the conformations are quite different. The degree of freedom is nearly the same as in a conventional MD simulation and it is possible to calculate energies of the system during the transition.

A well-suited application of the method is the T ↔ R transition in insulin. Its most striking aspect is the refolding of the N-terminal octapeptide of the B chain. The T and R state were defined by high resolution X-ray analysis of four crystal modifications of hexameric zinc insulin [13–16]. The protomer composition is T_6 in rhombohedral 2 Zn insulin, T_3R_3 in rhombohedral 4 Zn insulin and R_6 in monoclinic insulin as well as in another rhombohedral form. It was first shown in our laboratory that these structural states are adopted also in solution [17, 18]. The $T_6 \rightarrow T_3R_3$ transformation is promoted by high concentrations of halide ions, the $T_6 \rightarrow R_6$ transformation by phenolic compounds [17–23].

The T and R structures are depicted in Figure 1. Residues B1-B8 are extended in T an helical in R. Much smatter but significant differences are also found in other parts of the molecule. They result from a displacement or rotation of whole segments, e.g. the A_N-helix and the A10-A12 extended chain, or from modification of the chain conformation as in the C-terminal B chain. Further details are described elsewhere [24, 25].

The T ↔ R transition in the hexamer can be undergone only concertedly by all subunits of a trimer. Sigmoid binding curves obtained in titration experiments with phenol are clearly indicating a positive cooperativity [26, 27]. Although the transition for a subunit in a hexamer will thus be influenced by the neighbour subunits, the present simulation was limited to the monomer. The reason was to choose an example as "simple" as possible for describing and exploring the potentialities of the method. The results can be compared with those of a recent simulation using the method of targeted energy minimisation (TEM). This approach has been applied to both, a monomer and a hexamer and has shown that the pathways are largely

similar [12]. Last *but* not least it is being taken into consideration that the transition might really occur also in the monomer, albeit as a rare event.

In this simulation method the role of the target structure is that of providing a constraint. For a conformational change as extensive as in the present case there is no way around a target bound constraint of some kind. Despite the directedness of the simulation there is enough flexibility to explore surrounding conformational space. From this point of view the method is capable of finding one or more pathways plausible with respect to energy barriers to be surmounted during the transition. It seems possible to test on the validity of the pathways experimentally by using the inherent information to conceive mutations presumed to enhance or inhibit the transition.

METHOD

Geometry

In the following we shall consider the transition of a molecule from an initial conformation I to a final conformation F. The conformations may be defined as a certain region in the neighbourhood of representative configurations x_I or x_F, respectively, which is thermically accessible without crossing appreciable barriers and hence is sampled in the course of time.

For a molecule consisting of N atoms each configuration is given as a vector $x = (x_1 \ldots x_{3N})^T$ containing the 3N cartesian coordinates of the position vectors of the $r_1 \ldots r_N$ of the individual atoms. The aim is to find one or several continuous pathways from x_I x_F which are representative of the actual transition from I to F at given temperature, pressure and environment.

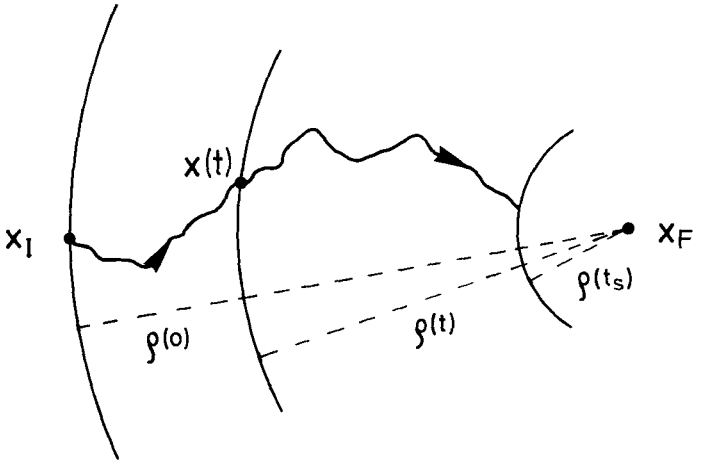

Figure 2 Two-dimensional projection of the conformational space onto a plane containing the initial and target configuration x_I and x_F representing the initial and final conformations I and F, respectively. By continously diminishing the distance ρ from the target x_F during the simulation (starting at $t = 0$ and ending at $t = t_S$), a path $\{x(t)\}$ is generated leading from x_I ($\rho(0)$) to a final configuration $x(t_S)$ with a given distance ($\rho = \rho(t_S)$) as small as possible. Note that the cartesian coordinates are otherwise free to move. The geometrical constraint is actually defined in a system of mass-weighted cartesian coordinates.

In close analogy to the method presented recently [12] intermediates x with a given distance π from the final configuration x_F are searched for, see Figure 2. The distance is defined by

$$\rho = |x - x_F| = \left(\sum_{i=1}^{3N} (x_i - x_{Fi})^2 \right)^{1/2} \tag{1}$$

i.e. all x with the same value of ρ are lying on a hypersphere around the final configuration. For monitoring the transition we perform a simulation of length t_S with a time-dependent distance $\rho = \rho(t)$ starting at $\rho(0) = |x_I - x_F|$ and shrinking to a final value $\rho(t_S)$ which is chosen as small as possible. It is clear that by using this strategy the molecule is forced to find a path from x_I to some final configuration close to x_F. At every $\rho > 0$ all 3N-1 remaining coordinates and thus the individual atoms are free to move in accordance with the force field and the environmental parameters so that the system can sample a variety of intermediate configurations. Nevertheless the method ensures the generation of pathways in which the distance from the final configuration is monotonously reduced. For a macromolecule the restriction of the number of coordinates to only one less will not seriously afflict the internal mobility as long as ρ is large enough. At decreasing ρ, however, the dynamic fluctuations are finally frozen and a static structure is obtained. By eq. (1), the transition to strongly reduced dynamics is expected to occur near $\rho = (3N\sigma^2)^{1/2}$, where σ is the mean thermal standard deviation per coordinate of the unconstrained molecule in the final state.

Centre-of-mass invariant Formulation

The investigation of a transition at a given temperature T and pressure P requires the application of a MD simulation technique which solves the classical equations of motion and also includes coupling to an external bath of constant T and P. For controlling ρ (which is intended to be varied later on) the following constraint was introduced:

$$\Phi(x) = (x - x_F)^2 - \rho^2 = 0 \tag{2}$$

which results in an additional constraining force

$$F^c = \lambda \, d\Phi/dx = 2\lambda(x - x_F) \tag{3}$$

where λ is an adjustable Lagrange parameter. It can be shown that even if the centres of mass (CM's) of x and x_F are the same, neither the position of the CM nor the orientation of the molecule are invariant under the influence of this force, which therefore causes translatory and rotatory fluctuations.

Translation can be avoided by choosing a slightly different constraint using mass-weighted coordinates. The original position vectors r_j are replaced by $r'_j = \mu_j r_j$, where $\mu_j = (m_j/\langle m \rangle)^{1/2}$, m_j is the mass of the respective atom, and $\langle m \rangle$ the mean atomic mass in the molecule. The vectors built up by the r'_j are denoted by x'. With the new coordinates the distance and the corresponding constraint are redefined by

$$\rho' = |x' - x'_F| = \left(\sum_{i=1}^{3N} (x'_i - x'_{Fi})^2 \right)^{1/2} \tag{4}$$

$$\Phi(x') = (x' - x'_F)^2 - \rho'^2 = 0 \tag{5}$$

For each atom j this yields a constraining force

$$F_j^c = \lambda \, d\Phi/dr_j = 2\lambda \, \mu_j(r'_j - r'_{Fj}) = 2\lambda \, \mu_j^2(r_j - r_{Fj}) \tag{6}$$

The total constraint force exerted on the molecule then vanishes as

$$F_{tot} = \sum F_j^c \propto \sum \mu_j^2(r_j - r_{Fj}) \propto \sum m_j(r_j - r_{Fj}) = 0 \tag{7}$$

if the CM's of x and x_F are the same. This proves that by application of the new constraint (5) the CM is kept constant and translations do not occur during the simulation when the initial and final configuration have the same CM. Rotation could be controlled by an additional constraint. However, it proved sufficient to stop rotation repeatedly after a certain number of time steps.

Leap Frog Algorithm with Constraint

The leap frog algorithm [28] which is equivalent to the Verlet algorithm [29] has been widely applied in MD simulations, and was modified to also include constraints for bond lengths [30]. An analogous treatment of constraint (5) is used here. For a single coordinate x_i, the new value at a time $t + \Delta t$ is calculated from the former coordinate and velocity according to

$$\begin{aligned} x_i = x_i(t + \Delta t) &= x_i(t) + \Delta t \, [\, (v_i(t - \Delta t/2) + \Delta t \, F_i(t)/m_i + \Delta t \, 2\lambda \, \mu_i^2(x_i(t) \\ &\quad - x_{Fi})/m_i)\,] \\ &= x_i^{\#} + \delta x_i \end{aligned} \tag{8}$$

(In this equation the terms simulating the coupling to the temperature and pressure bath are omitted.) $x_i^{\#}$ is the value computed without constraint ($\lambda = 0$) as delivered immediately by the program, and δx_i the correction to be calculated by means of the constraint (5). By multiplying with the weighting factors μ_i corresponding to the coordinates i (for the sake of simplicity, the μ_i are given the coordinate index i here) and inserting in (5), the parameter λ is eliminated and one obtains a correction

$$\delta x_i = \gamma(x_i(t) - x_{Fi}) \tag{9}$$

where γ is given by a quadratic equation

$$\gamma^2(x'(t) - x'_F)^2 + 2\gamma(x'(t) - x'_F)^T(x'^{\#} - x'_F) + \Phi(x'^{\#}) = 0 \tag{10}$$

At sufficiently small time, steps eq. (10) yields two reat values for γ. This can be seen by inserting $\Phi(x'^{\#})$ from (6) and using the fact that $x'^{\#}$ approaches $x'(t)$ in the limit $\Delta t \to 0$, see eq. (8). In order to obtain a quasi continuous trajectory the γ with the lowest absolute value has to be taken throughout the simutation. γ becomes zero and the correction vanishes when the constraint is already satisfied by $x'^{\#}$, $\Phi(x'^{\#}) = 0$, as it must be.

COMPUTATIONAL PROCEDURE

As initial and final geometry for the simulation of the T → R and R → T transition, respectively, the X-ray coordinates of molecule 2 (Chinese nomenclature) of rhombohedral porcine 2 Zn and 4 Zn pig insulin (G. Dodson, personal communication) were taken. The resolution of both structures was 1.5 A.

The GROMOS program package was used to perform the initial energy minimisations (EM) and the MD simulations [31]. The algorithm of this method was included by implementing another subroutine SHIFT.

The monomer consists of 488 atoms, when the united atoms approach is applied in which only polar hydrogens are explicitly taken into account. The standard GROMOS parameter set for vacuum conditions with partial charges for the charged atoms was used. Before starting the MD simulations both X-ray structures were subjected to energy minimisation using the conjugate gradient method without applying SHAKE [32]. The cut-off radii for the Lennard-Jones interactions are $R_{LJ} = 1.0$ nm and for the Coulomb interactions $R_C = 1.2$ nm, respectively. Minimisation was continued until the potential energy change was less than 0.1 kj/mol per step.

Before used in the simulation the coordinates of the R state were superimposed onto those of the T state. The residual distance $\rho'(0)$ between the superimposed structures was 14.89 nm.

Three MD simulations of 50, 100 and 200 ps were performed in both the T → R and the R → T direction. SHAKE was applied in the MD simulation to keep the bond lengths constant. The time step for integration of the equations of motion was 2 fs. The pair list of long range interactions was updated every 40 fs. The simulation was started at a temperature of 300 K and the velocities were taken from the corresponding Maxwell distribution. The system was coupled to a temperature bath with a relaxation time of 0.01 ps. The rotation of the molecule was stopped every 100 fs. The output frequency to analyse the simulations was 0.5 ps.

The parameter $\rho'(t_s)$ indicating the residual distance from the final structure, where the simulation is stopped (see Figure 2), is set to 0.89 nm, corresponding to a total root mean square (rms) positional difference of 0.4 A.

The resulting simulation velocity $\Delta\rho'/\Delta t$, which is equal to $\rho'(0) - \rho'(t_s)/t_s$, amounts to 0.28, 0.14 and 0.07 nm/ps in the 50, 100 and 200 ps simulations, respectively.

The calculations were performed on an IBM 3090, with an approximate CPU time of 4.5 minutes per ps, which is nearly equal to the time of unmodified MD simulation. For graphical and numerical analysis, respectively, the programs ACAMOD [33], WHATIF [34] and SIMLYS [35, 36] were used.

RESULTS AND DISCUSSION

The Potential Surface under the Trajectory

The potential energy is plotted as a function of ρ' for the 200 ps T → R and R → T simulations in Figure 3a and 3b, respectively. Both curves show fluctuations of +/− 100 kJ/mol and a very shallow minimum. The dependence of the average potential and total energy on the simulation velocity $\Delta\rho'/\Delta t$ can be gathered from Table 1.

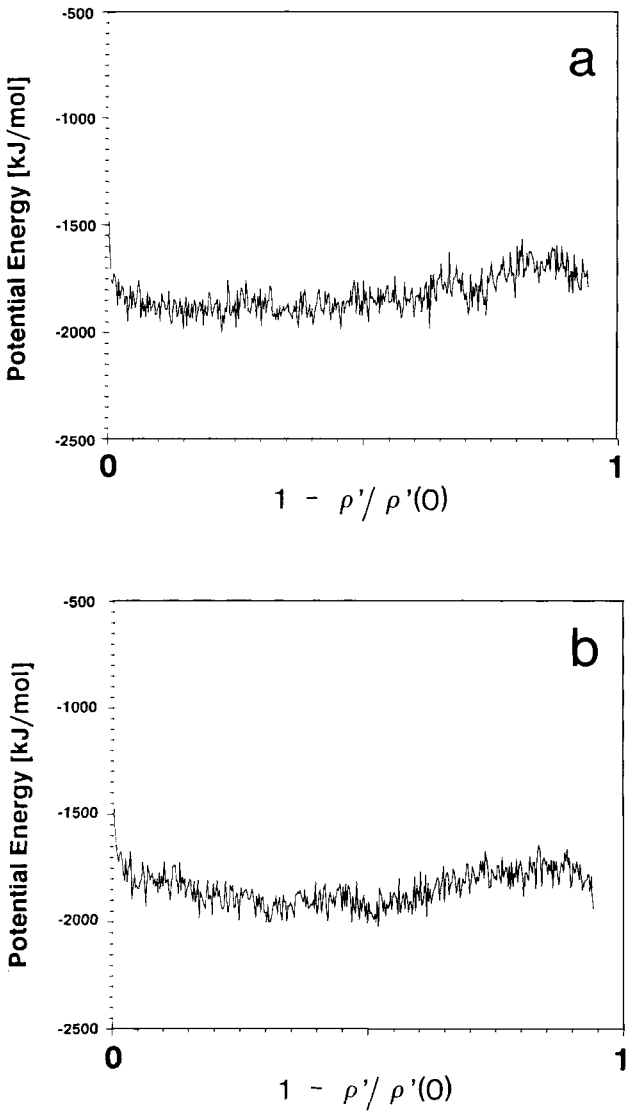

Figure 3 Potential energies as a function of ρ' in the two 200 ps simulations of the T → R (a) and R → T (b) transition. $\rho'(0)$ corresponds to the mass weighted rms distance between x_I and x_F and amounts to 14.9 nm.

The potential energy as well as the total energy decrease when the duration of the simulation is increased. Apparently in more time the molecule can explore more of the conformational space for lower potential energy barriers.

There is not much difference between the two pathways, although the energies for the R → T direction are slightly below those for T → R.

A crude comparison of these energies with those obtained in the previous TEM

Table 1 Averaged energy values and their rms deviations (in brackets) for three transformation velocities.

	T → R		R → T	
$\Delta\rho'/\Delta t$ nm/ps	⟨Epot⟩ kJ/mol	⟨Etot⟩ kJ/mol	⟨Epot⟩ kJ/mol	⟨Etot⟩ kJ/mol
0.28	−1745 (101)	−560 (100)	−1755 (88)	−570 (85)
0.14	−1805 (83)	−621 (81)	−1789 (66)	−605 (62)
0.07	−1818 (90)	−634 (87)	−1842 (87)	−658 (84)
$\Delta\rho'/\Delta t$ nm/ps	⟨Epot⟩ kJ/mol	$\langle U_0 \rangle$ kJ/mol	⟨Epot⟩ kJ/mol	$\langle U_0 \rangle$ kJ/mol
0.28	1185	−2930	1185	−2940
0.14	1184	−2989	1184	−2973
0.07	1184	−3002	1184	−3026

$\langle U_0 \rangle$ obtained by the TEM method: −2899 kJ mol^{-1} (T → R) and −2859 kJ mol^{-1} (R → T) [12].

simulation is possible after subtraction of the kinetic energy from the potential energy

$$U_0 = \langle E_{pot} \rangle - \langle E_{kin} \rangle. \tag{11}$$

(This expression would yield the exact zero energy in U_0 in an harmonic potential.) In TMD the imposed constraint is more restrictive than in the TEM simulation due to its application in every MD step. Nevertheless the average values of U_0 of the former method are below those of the latter. It is known that energy minimisation can relax large localised stresses quickly but may relax delocalised stresses very slowly [37]. In contrast the MD simulated thermal motions allow the structure to resolve steric conflicts by a process that is akin to annealing [10]. The steric stress is faster dissipated throughout the structure which leads to a lower level of total potential energy.

The Paths through Conformational Space

The pathways obtained in the 200 ps simulations were selected for (a more) detailed analysis because their potential energy level is minimum. Although the pathways for the forward and backward direction have similar average energy and energy profiles, this does not necessarily mean that they overlap. Figure 4 shows the trajectories of the geometric centre of Phe^{B1} in the forward and backward transition. The traces exhibit appreciable differences between the two pathways. It occurs that there is accumulation around single spots indicating that the transition hesitates to progress presumably because of steric hindrance. Although one must be cautious with generalizing conclusions drawn from the movement of a single residue, appreciable differences between the two pathways remain, and moreover the situation is similar to that seen in the TEM simulation.

In Figures 5 and 6 the forward and backward transitions are illustrated by series of selected snapshots. The distances between the snapshots correspond, but the R → T transition has been reversed in order to make a parallel comparison possible.

The T → R transition starts with a swivelling motion of the 4 N-terminal residues of the B chain along parts of the A chain, which in the further course of the simulation seizes the whole segment BI-B8. When the B chain N-terminus points in the

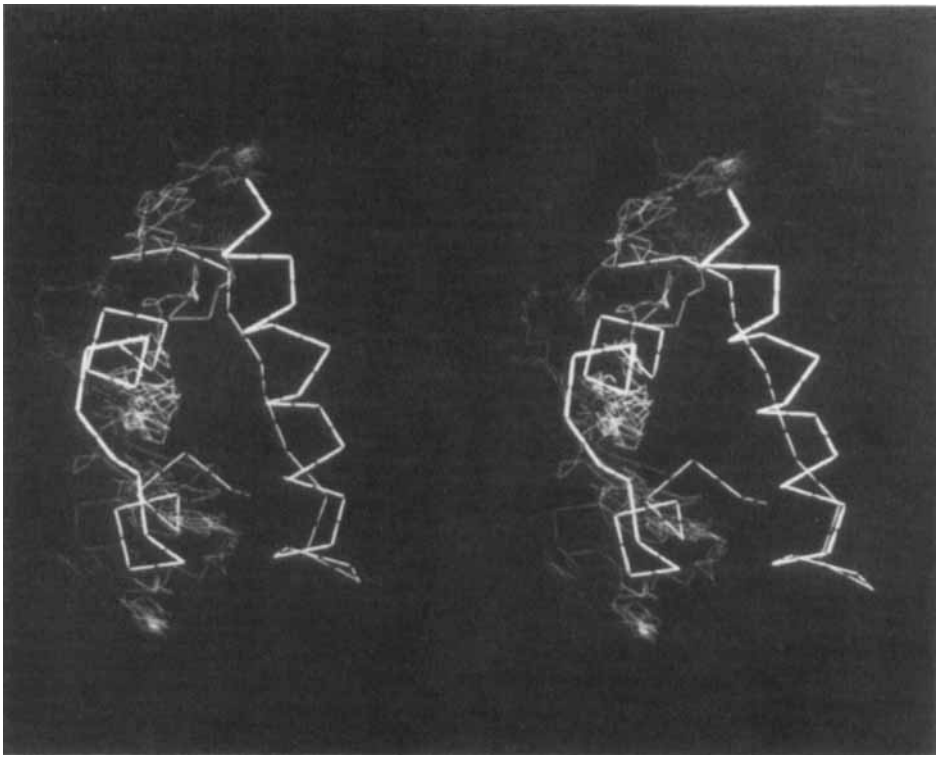

Figure 4 Trajectory of the geometric centre of Phe^{B1} in the simulated T → R (yellow) and R → T (green) transition in stereo. The C_α-structures of T and R are underlaid in red and blue, respectively. (See Colour Plates)

direction of the axis of the central B helix it is stretched again and the helix folding actually begins.

The first event in the R → T transition is a bending of the B1-B19 helix involving the main chain dihedral angles of B8, B9 (see also Figure 9) and B10 (not depicted). Then the helix kinks in this region and the whole N-terminal segment swivels in the direction of its final position. The N-terminal helix is maintained during the first part of this motion. Thereafter unwinding starts from B1.

As already seen in the TEM simulation, the refolding of the N-terminal B chain is accompanied by an intermediate displacement of the A_N helix and the inter-helical segment A10-A12. The closest interchain contact involves Ile^{A10} and Asn^{B3}. The trajectories of the geometric centres of these residues exhibit much more fluctuations than that of Glu^{A15} which is not affected by the refolding events (see Figure 7). Interestingly in an NMR study on des-pentapeptide$^{B26\text{-}B30}$ insulin (DPI) [38] many NOEs were found between Ile^{A10} and Asn^{B3}, Gln^{B4}, His^{B5} and Leu^{B6}, respectively. These contacts are not expected from the crystal structure and may represent an average of fast movements of the N-terminal B Chain.

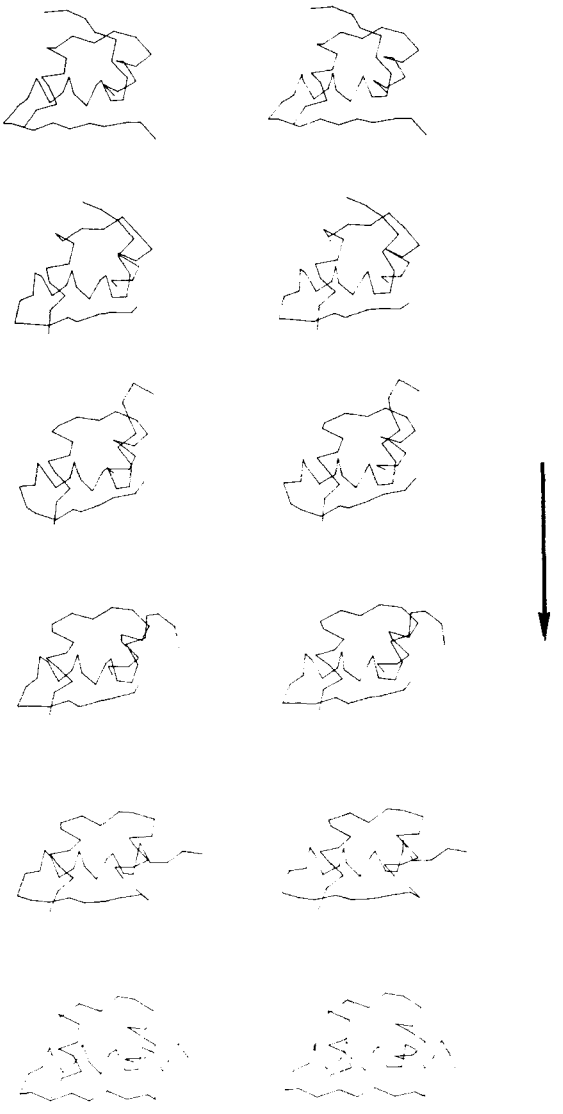

Figure 5 Stereo drawings of the C_α-structures at 0, 20, 60, 100, 140, 180 ps (from top to bottom) during the T → R simulation. The corresponding ρ' values are 14.89, 13.89, 10.69, 7.89, 5.09, 2.29 nm, respectively. All structures are super-imposed on the X-ray structure of T for optimum fit to the central B helix (B9-B19).

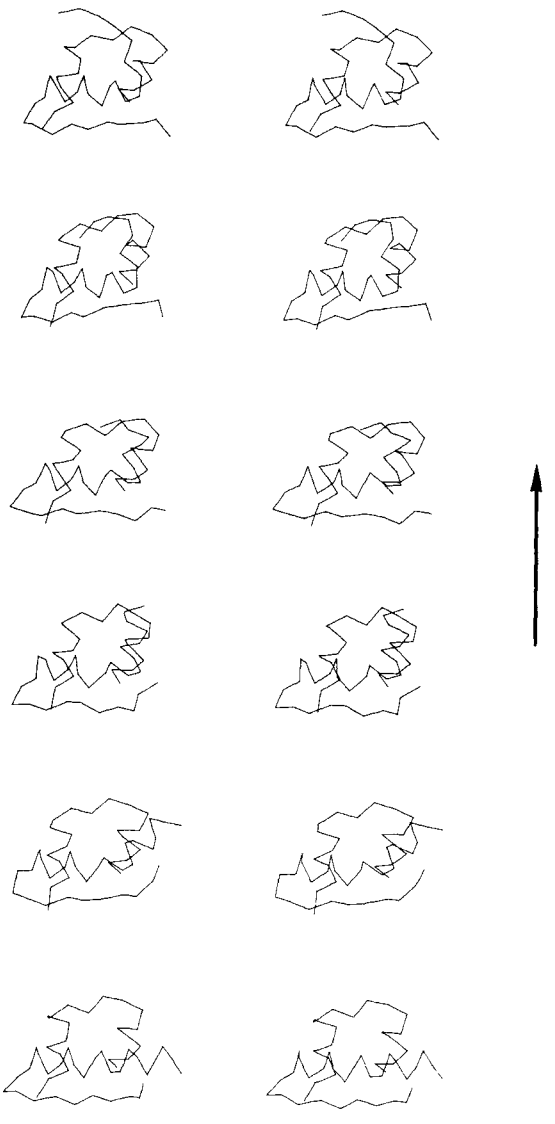

Figure 6 Stereo drawings of C_α-structures at 0, 20, 60, 100, 140, 180 ps (from bottom to top) during R → T simulation. The arrangement of the structures is reversed compared to Figure 5 in order to make a roughly parallel comparison possible. (ρ' values and fit as for Figure 5.)

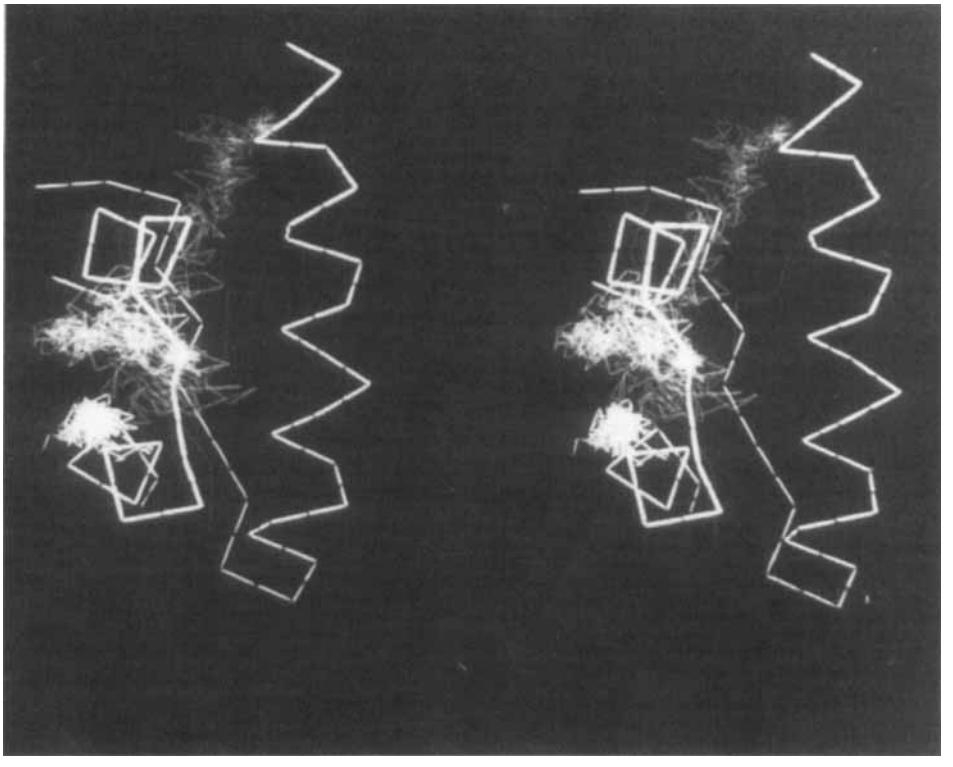

Figure 7 Stereo tracing of the trajectories of the geometric centres of Asn^{B3} (yellow), Ile^{A10} (green) and Glu^{A15} (violet) in the 200 ps T → R simulation. The C_α-structures of T and R are underlaid in red and blue, respectively. (See Colour Plates)

Φ, Ψ *Angles of the N-terminal B Chain Residues*

The secondary structural change is illustrated by the Φ, Ψ main chain dihedral angles as a function of ρ' Figures 8 and 9. As to expected the amplitudes of the fluctuations are largest for Ψ_{B1}. Abrupt changes are more frequent than gradual ones. Both, simultaneous as well as independent changes of Φ and Ψ are observed. Sometimes changes of Φ_i and Ψ_{i-1} are collelated (for example Φ_{B5}/Ψ_{B4} or Φ_{B7}/Ψ_{B6} in T → R and Φ_{B4}/Ψ_{B3}, Φ_{B7}/Ψ_{B6} or Φ_{B8}/Ψ_{B7} in R → T) which are known to be energetically favourable and not to have much effect on the overall chain conformation [39]. In the T → R direction (Figure 8) the helical values of R are adopted only in the last third of the transition with the angles at B6 preceding a bit and those at B7 and B8 coming a bit later. The earliest changes are seen for Ψ_{B2} and Φ_{B4} which seem to reflect the swivelling motion of the N-terminal tetrapeptide mentioned above.

In the R → T direction (Figure 9) the earliest event seen is a temporary change of Φ_{B9} which may be related to a slight concomitant perturbation of Φ_{B8} and Ψ_{B8} and, hence, to the bending of the B1-B19 helix mentioned above. The N-terminal helix,

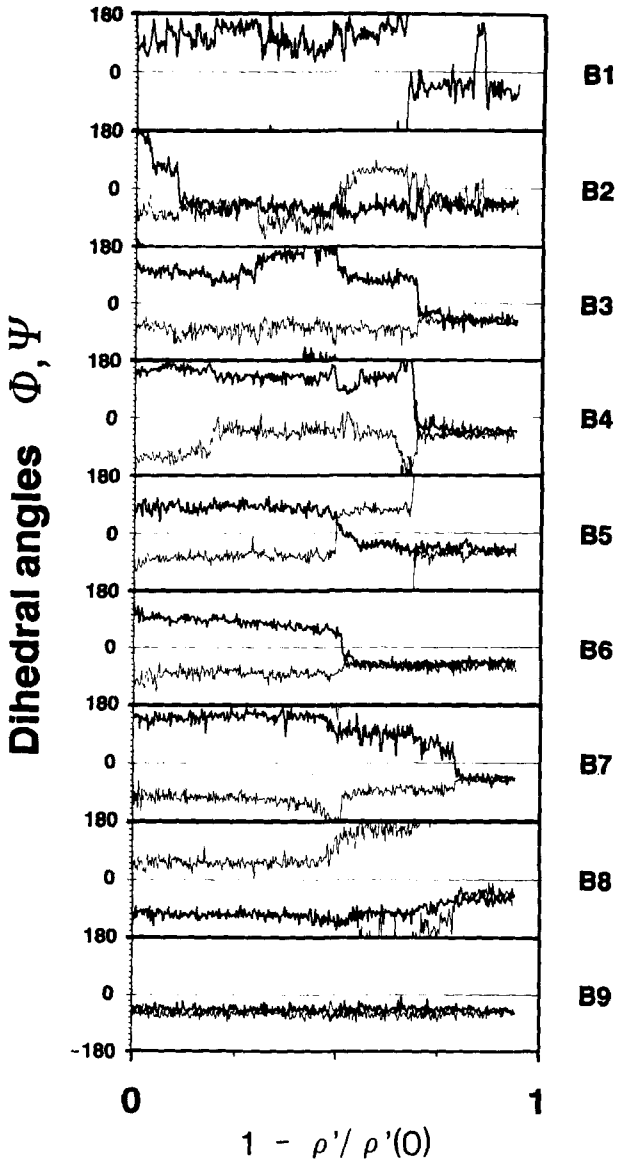

Figure 8 The 9 N-terminal main chain dihedral angles Φ (light lines) and Ψ (heavy lines) of the B chain as a function of ρ' in the R → T simulation.

however, is preserved during half of the simulation time. Then gradual changes start at B5 and B7 which coincide with an abrupt change of Φ_{B2} and Ψ_{B2}. Only after three quarters of the simulation time the angles at B1-B5 adopt the values characteristic of T, those at B6-B8 even at the very end. This is a reflection of roughly sequential stretching.

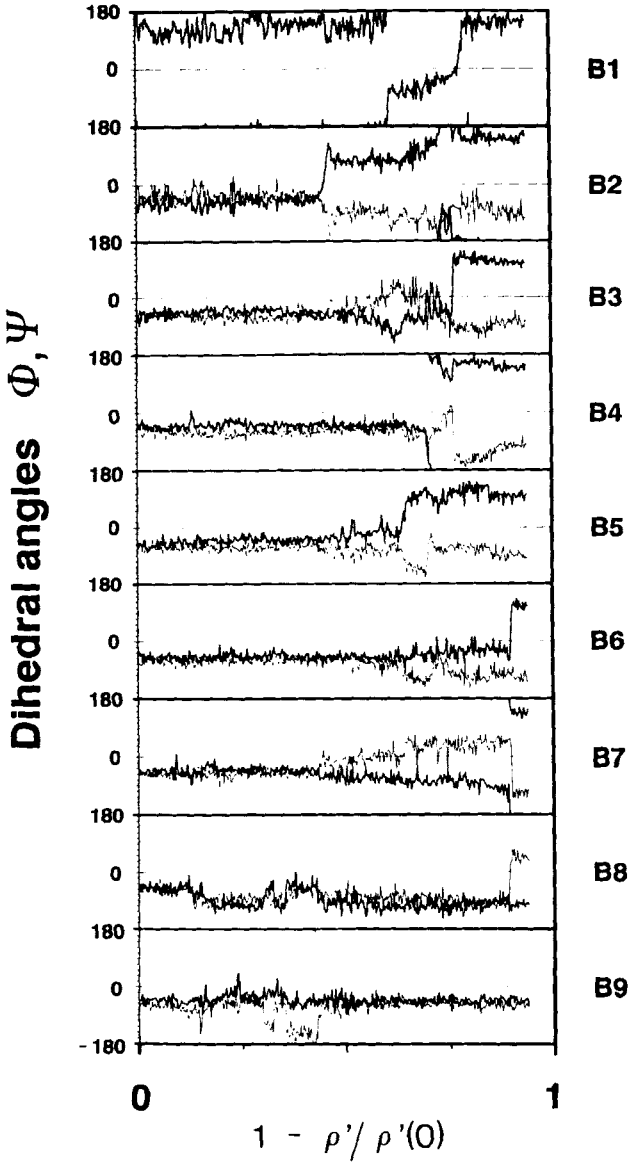

Figure 9 The 9 N-terminal main chain dihedral angles Φ (light lines) and Ψ (heavy lines) of the B chain as a function of ρ' in the R → T simulation.

CONCLUSIONS

In this paper we have presented a new method called TMD for simulating a conformational transition from a known starting structure to a known target structure of a macromolecule by means of MD at finite temperature and combined with a geometrical constraint. In contrast to other constraint MD methods [40,41] no information of preceding pathway calculations [42] were included. The algorithm was applied to the T ↔ R structural transition of insulin which is representative of extensive conformational changes in proteins. Our results have shown that the method is indeed able to find reasonable pathways between the input structures which, however, depend on the direction of the transition. TMD yields pathways whose mean potential energy is considerably lower than that recently obtained with the TEM method.

It is clear from both theoretical and experimental findings that a unique pathway cannot be expected to exist for a conformational transition in a large molecule. The potential energy surface of a protein posseses a large number of local minima separated by low barriers which are quickly crossed at room temperature [43]. There may also be different larger barriers the system can cross during the same process, which then results in a distribution of activation energies [44]. As a consequence, one has to consider a distribution of different pathways which depends on temperature, pressure and other environmental parameters.

In contrast to any approach using energy minimisation, TMD is certainly a method to find pathways that is independent of the irrelevant fine structure of low energy barriers and takes into account the parameters mentioned. By variation of the input structures and velocities different pathways can be sampled leading to a model of large scale motions during the process considered. There are two features which restrict the variety of pathways TMD can find in this way. In the first place, it selects only pathways with monotonously decreasing distance from the target structure. In the second place, there is no global criterion which guarantees that the mean or maximum energy is minimised along a pathway. The MD step onto the next hypershere rather tends to find a configuration which can be reached from the previous one at the minimum cost of energy, thus fulfilling a rather local criterion. Both features contribute to some kind of directedness in the method which may explain the different pathways found for the forward and backward transition. If there were a pronounced optimum pathway, however, it might rather be found in longer simulation times and at higher temperatures which enable the system to explore a greater part of the configurational space.

Concerning insulin, the present calculations are another step towards an understanding of the T ↔ R transition. For each direction different pathways and sequences of events were obtained, but no preference is possible by energy criteria at the present stage. We are also aware of the reservations to be made with respect to solvent, hexamer environment, zinc ions and transforming agents neglected in this monomer simulation. Stepwise adaptation of the simulation conditions to reatity as well as experimental validation of the simulated pathways will engage our activity in the future.

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