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Reversible Peptide Folding in Solution by Molecular Dynamics Simulation

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Long-standing questions on how peptides fold are addressed by the simulation at different temperatures of the reversible folding of a peptide in solution in atomic detail. Molecular dynamics simulations correctly predict the structure that is thermodynamically stable at 298 K, irrespective of the initial peptide conformation. The rate of folding and the free energy of folding at different temperatures are estimated. Although the conformational space potentially accessible to the peptide is extremely large, very few conformers (10^1 to 10^2) are significantly populated at 20 K above the melting temperature. This implies that the search problem in peptide (or even protein) folding is surmountable using dynamics simulations.

Keywords: peptide folding; molecular dynamics; β-peptides; free energy; GROMOS

Introduction

Understanding the process of peptide folding is a critical first step toward understanding protein folding and would greatly facilitate the *de novo* design of peptides with predetermined structures and properties for biotechnological use (Karplus & Shakhnovich, 1992; Borman, 1997). Peptides are, however, highly flexible and in solution can adopt a variety of conformations depending on the temperature and solvent conditions (Marshall *et al.*, 1995). Determining the structure of small peptides in solution experimentally is non-trivial. This makes methodology to predict how peptides fold in solution of fundamental importance in structural (bio)chemistry. In principle, the process of peptide folding could be simulated directly on a computer. It had been commonly assumed, however, that the volume of conformational space accessible, even to a small peptide, meant that simulating the folding of a peptide under realistic conditions was not, and for the foreseeable future would not be, possible. Here, we demonstrate that this is not true by simulating the reversible folding of a β-heptapeptide in methanol at a series of temperatures.

Theoretical approaches to predict the (stable) folded structure of a peptide or, which is more difficult, the process of peptide folding fall into three categories; statistical approaches, conformational search methods and dynamics simulation (Troyer & Cohen, 1991). Statistical approaches relate amino acid sequence to known three-dimensional structures and are reasonably successful at predicting elements of secondary structure (Schulz, 1988; Thomas & Dill, 1996). They require, however, knowledge of the structures of related sequences, cannot predict novel folds and provide no information on the mechanism of folding. Statistical methods also attempt to assign to the system a single, unique fold. This is, in general, a poor representation of the situation in solution (Marshall *et al.*, 1995). Conformational search methods can, in principle, yield the relative population of different conformers. A range of conformations is generated and an energy function is used to discriminate between them (Leach, 1991; Pedersen & Moult, 1997). However, the size of the conformational space accessible, even for small peptides, precludes any form of systematic search at atomic resolution (Karplus & Shakhnovich, 1992). Dynamics simulation methods, such as molecular dynamics (MD), have been used to characterize particular folded states in solution (Tobias *et al.*, 1991; Hermans, 1993; Kovacs *et al.*, 1995; Constantine *et al.*, 1996), but it is generally assumed that simulations involving physical force-fields, atomic degrees of freedom and explicit solvent cannot be used to predict peptide folding in solution. The tendency has been, instead, to turn to simplified models or represen-

Abbreviations used: MD, molecular dynamics; RMSD, root-mean-square deviation.

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tations (Karplus & Sali, 1995; Sung, 1994; Sung & Wu, 1996; Mohanty *et al.*, 1997; Kolinski & Skolnick, 1994; Vieth *et al.*, 1994; Ripoll & Scheraga, 1988; Yue & Dill, 1996). Unfortunately, none of these simplified approaches has met with clear success. The fundamental challenge is to reproduce the free-energy surface of the molecule in solution with sufficient fidelity. Most problematic, if not impossible, is the incorporation of effects due to solvent entropy in an implicit model. Simplified models allow greater sampling of conformational space but sample many regions with inappropriate weights.

The basic assumption that it is not possible to simulate the folding process of a peptide in solution with atomic detail has not been tested directly. The relevant question is not the volume of the accessible conformational space, but the frequency of interconversion between sets of folded and unfolded conformations (Dill & Chan, 1997; Shortle *et al.*, 1996; Eaton *et al.*, 1996). If the frequency of interconversion is high on the MD timescale, and equilibrium distribution between folded and unfolded conformations can be simulated and the dominant species in solution at a given temperature determined directly.

Here, we present a series of MD simulation studies on the folding of a β-heptapeptide (H-β-HVal-β-HAla-β-HLeu-(*S*,*S*)-β-HAla(αMe)-β-HVal-β-HAla-β-HLeu-OH: Seebach *et al.*, 1996b) in solution (Figure 1). This non-natural peptide, composed of β-amino acid residues (-HN-HC$^{\beta}$R-H$_2$C$^{\alpha}$-CO-, R being the side-chain) forms a stable (*M* or left-handed)-3$_1$-helix (Figure 2A) in methanol (Seebach *et al.*, 1996b). β-Peptides have recently attracted much attention due to their potential use as non-degradable peptide mimetics and the possibility to tune the conformation of the peptide by altering the side-chain composition (Seebach *et al.*, 1996a,b; Hinterman & Seebach, 1997; Apella *et al.*, 1996, 1997; Borman, 1997; Koert, 1997; Iverson, 1997). To investigate the ability of MD simulation to directly predict the folding of a given peptide, this system has several desirable properties. First, it has been demonstrated that, using the GROMOS96 simulation program package and force-field (van Gunsteren *et al.*, 1996), simulations of this β-heptapeptide in methanol satisfy all available experimental NMR data without the inclusion of artificial restraints (Daura *et al.*, 1997). Second, although the β-heptapeptide adopts a well-defined stable fold at 298 K, the system does not represent a trivial folding problem. Each β-amino acid has three backbone rotatable bonds compared to two for an α-amino acid, making the conformational search problem roughly equivalent to that of an α-decapeptide. Third, as the force-field has not been developed for β-amino acids, there can be no suspicion that the force-field might have been artificially adjusted to favour the experimental fold. Finally, methanol is, like water, a strongly hydrogen bonding solvent. Methanol has, however, a lower density than water, making it a computationally less expensive solvent in which to simulate folding.

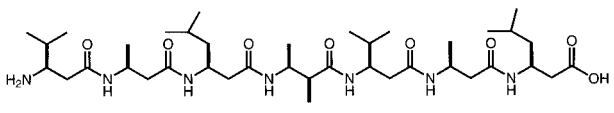

Figure 1. Structural formula of the β-heptapeptide studied. Note, in the simulations both end groups were protonated in line with experimental data.

Results and Discussion

The study is comprised of four parts. First, to demonstrate that the experimentally determined fold was in fact stable within the force-field at 298 K, a simulation of 50 ns in methanol was performed starting from the experimentally determined folded structure shown in Figure 2A (Daura *et al.*, 1997). Second, three simulations, each of 50 ns, were performed at progressively higher temperatures, 340 K, 350 K and 360 K, to examine how the relative populations of the folded and unfolded conformations depend on temperature. The simulations at 340 K and 350 K were started from the folded conformation (Figure 2A). The simulation at 360 K was started from an extended (all backbone dihedral angles set to 180°) conformation shown in Figure 2B. This was to avoid biasing the simulation toward the folded conformation. Third, a simulation at 340 K starting from the extended conformation (Figure 2B) was performed to demonstrate that the peptide could be folded from an arbitrary starting structure under (temperature) conditions where the 3$_1$-helical fold is thermodynamically stable. Fourth, 14 annealing simulations (0.75 ns each), starting from different unfolded conformations extracted from a simulation at 400 K (Daura *et al.*, 1997), were performed to investigate whether the peptide readily folds from a random starting structure to the 3$_1$-helical conformation on cooling to 300 K.

Reversible folding

Figure 3A and B shows the root-mean-square atom positional deviation (RMSD) from the 3$_1$-helical (or folded in our nomenclature) starting structure as a function of time for all main-chain atoms in residues 2 to 6 and residues 1 to 7, respectively, for the simulation at 298 K. The central region of the helix was stable at 298 K in the simulation. However, one event of unfolding and refolding was observed between times 38 ns and 40 ns. The average RMSD of the backbone atoms of residues 2 to 6 is of the order of 0.05 nm. The two terminal residues were more mobile and dominate the RMSD of the entire chain. From an examination of structures extracted from the trajectory it is clear that conformations that have an RMSD from the experimental (3$_1$-helical, folded) structure of less

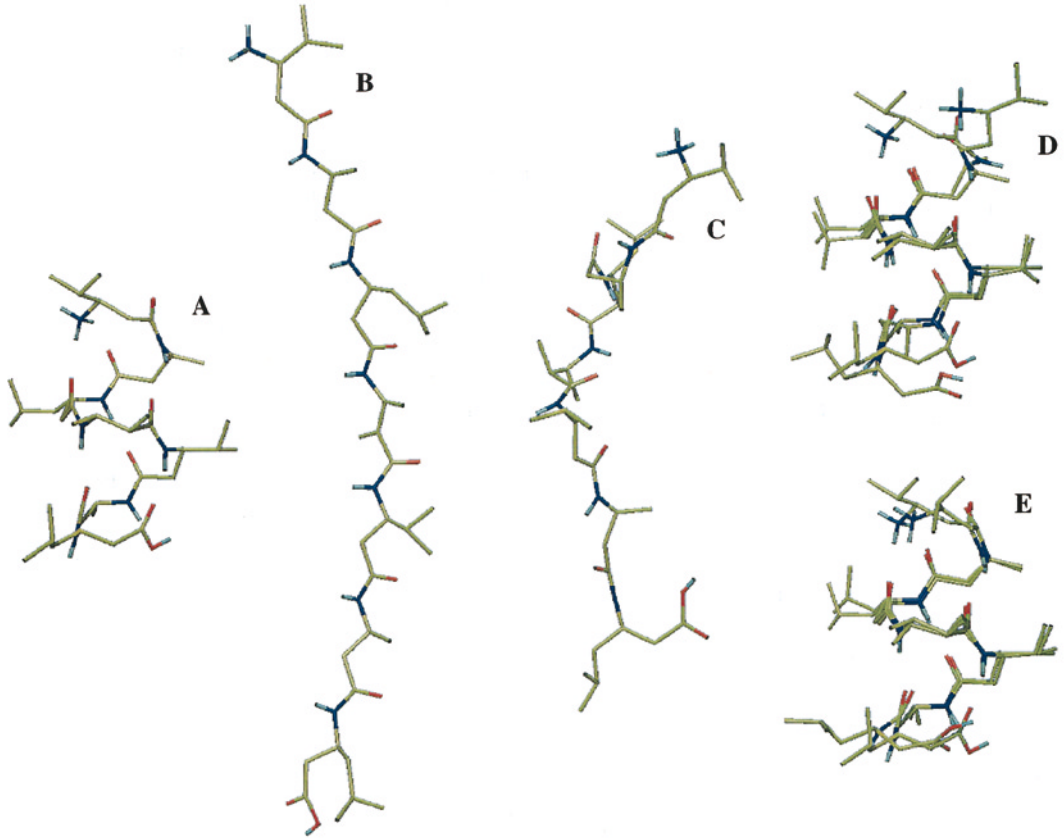

Figure 2. Conformations of the β-heptapeptide shown in Figure 1 in methanol. A, The (3_1-helical) reference structure determined experimentally (Seebach *et al.*, 1996b) by NMR at 298 K; B, an extended conformation generated by setting all backbone dihedral angles to 180° (the backbone atom positional RMSD to the (3_1-helical) reference structure is 0.52 nm (residues 2 to 6) and 0.72 nm (residues 1 to 7)); C, an initial structure used for annealing simulations (backbone RMSD of 0.25 nm (residues 2 to 6) and 0.44 nm (residues 1 to 7)); D, a superposition of the experimental reference structure and a structure extracted from the simulation at 360 K after 14.4 ns (backbone RMSD of 0.05 nm (residues 2 to 6) and 0.14 nm (residues 1 to 7)); E, a superposition of the experimental reference structure and a structure extracted from the simulation at 340 K starting from the extended structure after 3.95 ns (backbone RMSD of 0.02 nm (residues 2 to 6) and 0.06 (residues 1 to 7)).

than or equal to 0.10 nm for the main chain between residues 2 and 6 and an RMSD of less than or equal to 0.20 nm for the whole main chain represent unequivocally the (*M*)-3_1-helix fold. These values are indicated by the broken lines in Figures 3 and 4.

The effective melting temperature of the peptide in the force-field is close to 340 K. Starting from the 3_1-helical folded conformation, at 340 K the helix partially unfolded and refolded twice during the first 1.7 ns (Figure 3C). It then completely unfolded and remained unfolded for the following 8 ns. After this, the peptide fully refolded and unfolded several times during the 50 ns. A backbone RMSD of more than 0.20 nm (residues 2 to 6) represents a clearly unfolded conformation. This is evident from Figure 2C, which illustrates a starting structure used for the later annealing simulations. At 340 K the system spends approximately half the time simulated in the folded state and half in the unfolded state, implying that the difference in free energy between the folded and unfolded states is essentially zero. Above the melting temperature the peptide is not constantly disordered but spends proportionally less time in the folded conformation. At 350 K the helix partially unfolded immediately after the temperature was raised (Figure 3D). The backbone RMSD rose to 0.21 nm (residues 2 to 6) at 0.39 ns, but rapidly returned to less than 0.05 nm shortly afterwards. The system continued to fluctuate around the folded conformation for the following 5 ns. After this, the peptide fully unfolded and refolded several times during the 50 ns.

Folding from an arbitrary conformation

In contrast to the simulations at 340 K and 350 K, the simulation at 360 K was started from a fully extended conformation (Figure 2B). This starting structure was chosen to avoid biasing the simulations toward the folded state. Within 0.01 ns, however, the backbone RMSD had fallen from 0.52 nm to 0.44 nm (residues 2 to 6) and within 0.1 ns the conformations sampled were (in terms of RMSD from the 3_1-helical folded confor-

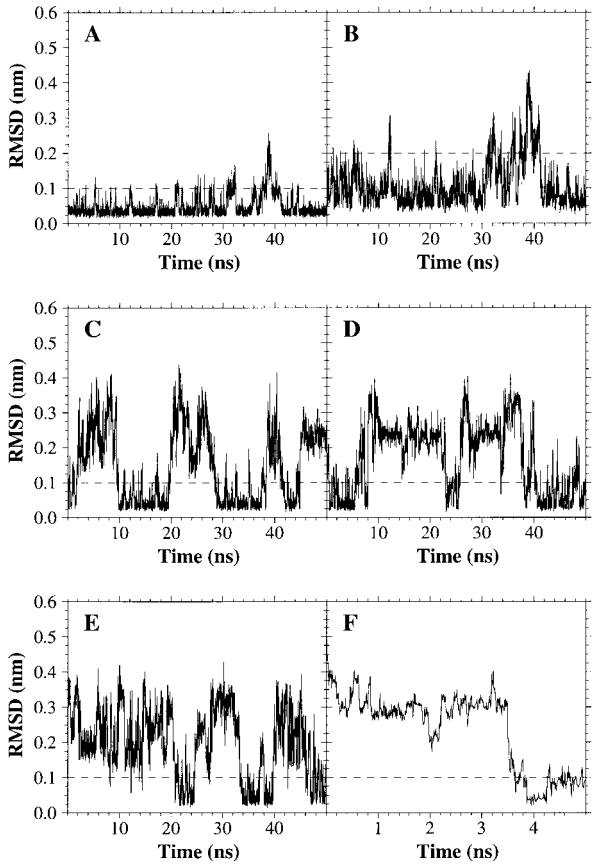

Figure 3. Backbone atom positional RMSD from the (3_1-helical) reference structure for residues 2 to 6 or 1 to 7 as a function of time in simulations at different temperatures and started from different initial configurations: A, RMSD for residues 2 to 6 at 298 K; B, RMSD for residues 1 to 7 at 298 K; C, RMSD for residues 2 to 6 at 340 K; D, RMSD for residues 2 to 6 at 350 K; E, RMSD for residues 2 to 6 at 360 K; F, RMSD for residues 2 to 6 at 340 K. Initial configurations: A to D, 3_1-helical structure (Figure 2A); E and F, extended structure (Figure 2B). Conformations with an RMSD below the broken line are unequivocally a 3_1-helix.

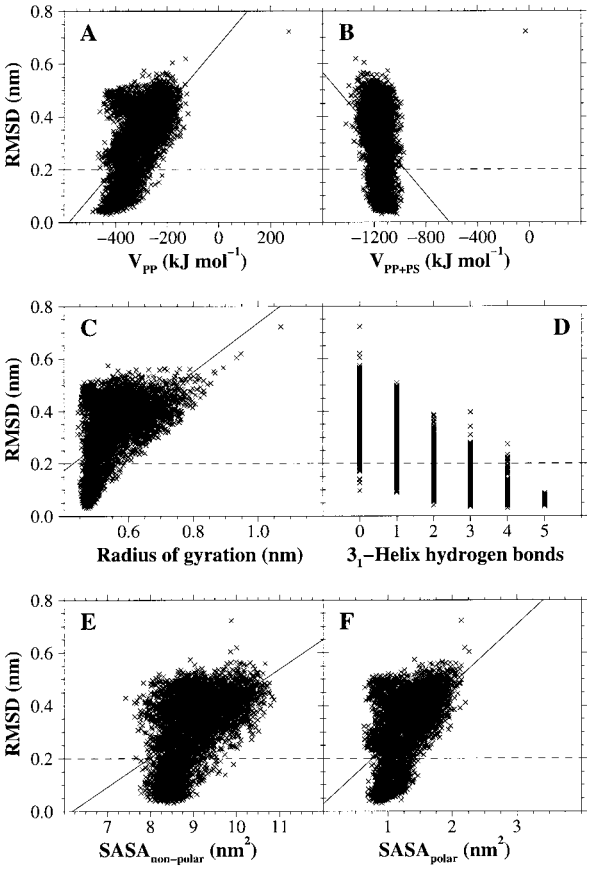

Figure 4. Correlation between the proximity to the folded conformation, measured as the backbone atom positional RMSD from the experimental (3_1-helical) structure for residues 1 to 7, at 360 K and: A, the total intra-molecular interaction energy of the peptide (correlation coefficient of 0.63); B, the total intra-molecular interaction energy of the peptide plus the energy of peptide-solvent interactions (correlation coefficient of −0.28); C, the radius of gyration of the peptide (correlation coefficient of 0.55); D, the number of 3_1-helix hydrogen bonds; E, the non-polar solvent-accessible surface area (correlation coefficient of 0.53); and F, the polar solvent-accessible surface area (correlation coefficient of 0.54). Conformations with an RMSD below the broken line are unequivocally a 3_1-helix.

mation) indistinguishable from unfolded conformations sampled in the simulations at 340 K or at 350 K (Figure 3E). At about 2.6 ns, the peptide adopted for the first time part of the helical conformation. Residues 4 to 7 folded into a 3_1-helix and the hydrogen bonds NH(4)-O(6) and NH(5)-O(7) were formed. Between 8 and 15 ns the peptide made a series of four distinct folding transitions where the backbone formed the 3_1-helix and the RMSD dropped below 0.1 nm (residues 2 to 6) for periods of the order of 0.03 ns (Figure 3E). The structure at approximately 14.4 ns superimposed on the experimental structure is shown in Figure 2D. The backbone RMSD between the two structures is 0.05 nm (residues 2 to 6). After the first 20 ns, the peptide fully refolded and unfolded several times, with the 3_1-helix being stable for periods of up to 4 ns.

Figures 3C to E demonstrate that it is possible to establish a distribution between folded and unfolded conformations in the simulations at or above the melting temperature, with more than one unfolding and refolding occurring at each temperature. From this, it is possible to ask which of the factors commonly considered important when studying peptide folding can be used to recognise the correctly folded state (Dill, 1990). The factors or properties examined were: (i) various energy terms, including the total potential energy of the system, the internal interaction energy of the peptide, and the energy of interaction between the peptide and the methanol solvent; (ii) the radius of gyration of the peptide; (iii) the presence of the hydrogen bonds that form the 3_1-helix; and (iv) the

non-polar and polar solvent-accessible surface area. In Figure 4 are plotted illustrative examples of the correlation between the folded conformation and some of the quantities examined, at 360 K. Because the properties considered are global, the backbone RMSD from the experimental structure for all residues 1 to 7 was used as a measure of the degree of folding. The simulation at 360 K sampled the widest range of conformations and in all cases shows a higher degree of correlation than the simulations at 340 K or 350 K. The single conformation with an RMSD from the experimental structure of 0.72 nm in each plot corresponds to the extended starting structure. The degree of correlation, however, even at 360 K, is very poor. The largest correlation coefficient, assuming a linear relationship between the RMSD and the quantity examined, was 0.63. None of the properties examined was by itself predictive of the folded state. Although conformations with a low RMSD also had, in general, a low radius of gyration, a low solvent-accessible surface area and a low intra-molecular energy, there were in all cases unfolded conformations with lower values. It might be thought that the total potential energy of the system, the enthalpy, should correlate with folding. In practice, the total potential energy is completely dominated by bulk solvent-solvent interactions. The change in enthalpy on folding is not detectable against this large background and no correlation between the total potential energy and the RMSD was observed. Of special interest are the hydrogen bonds. A hydrogen bond was considered to exist if the proton-acceptor distance was less than 0.25 nm and the donor-proton-acceptor angle was greater than 135°. As can be seen in Figure 4D, some of the conformations closest to the experimental structure have none of the five possible 3_1-helix hydrogen bonds present. Certainly, if four or more of the five possible hydrogen bonds are present, the helix is completely formed. However, the formation of 3_1 hydrogen bonds is apparently not a necessity in the folding process, and does not drive helix formation. This does not mean that these hydrogen bonds play no role in stabilizing the helix once formed. The three central 3_1-helical hydrogen bonds were highly persistent at room temperature (Daura *et al.*, 1997) and in those periods of time at 340 K, 350 K and 360 K in which the 3_1-helix was stable. In terms of the folding mechanism, this suggests that the formation of the hydrogen bonding network is the last step in the stabilization of the helix.

Figure 3E demonstrates that at 360 K it is possible to start from an extended conformation and sample conformations that are very close to the conformation stable at 298 K within 50 ns of simulation. It is also possible to fold the peptide from an extended structure under conditions where the 3_1-helix is the thermodynamically most stable conformation. Figure 3F shows the backbone RMSD (residues 2 to 6) from a simulation at 340 K started from the extended structure shown in Figure 2B. The backbone RMSD falls very rapidly (within 0.05 ns) from an initial value of 0.52 nm (residues 2 to 6) to a range indistinguishable from the unfolded states sampled in simulations started from the folded conformation. By 4 ns the peptide is completely folded (Figure 2E). This shows that high temperature (i.e. above the melting temperature) is not required to surmount possible energy barriers when folding from the extended structure and demonstrates that the reversible folding at 340 K evident in Figure 3C is independent of the starting structure.

Annealing simulations

The final part of this study involved the use of simulated annealing to investigate whether the peptide could be folded to the native conformation from a random starting structure in a short simulation time. Starting from seven randomly chosen structures with a radius of gyration greater than 0.6 nm, the system was cooled from 400 K to 300 K in a series of 20 K steps every 0.05 ns or 10 K steps every 0.025 ns, followed by 0.5 ns of simulation at 300 K. Although the annealing time was very short compared with the sampling shown in Figure 3A to E, the peptide was observed to fold to a 3_1-helix in two of the 14 simulations. For example, starting from the initial structure shown in Figure 2C, which had a backbone RMSD of 0.25 nm (residues 2 to 6) and a radius of gyration of 0.74 nm, the largest RMSD and radius of gyration of the seven starting structures used, the peptide folded rapidly forming the 3_1-helix within 0.09 ns while the temperature was at 360 K. The 3_1-helix remained stable as the system was further cooled. Folding into a 3_1-helix was not simply a consequence of the initial structure. The two successful annealing runs involved different starting structures and different annealing protocols. Such rapid annealing simulations cannot, however, be considered to predict the thermodynamically stable fold. The system became trapped in 12 out of 14 attempts and without an objective target function it would not be possible to determine which, if any, of the conformations found is actually thermodynamically stable.

Lessons for peptide folding

What lessons do these simulations teach for peptide folding studies? They demonstrate that using a physically based force-field, atomic degrees of freedom and explicit solvent it is possible to simulate the folding of a peptide from a random conformation to the experimentally observed conformation under reversible conditions. The transition from a totally extended conformation to the 3_1-helix required less than 10 ns both at 340 K and 360 K. The actual transition from an unfolded to a folded conformation was very rapid, of the order of 0.05 ns. The maximum lifetime observed

for the 3_1-helix at 340 K was of the order of 10 ns, the same observed for the unfolded state. Nevertheless, 50 ns of simulation yielded insufficient statistics to reliably determine the average lifetime of the folded and unfolded conformations at the three different temperatures for the β-heptapeptide. Experimental studies on the folding of an alanine-based α-peptide in water have suggested a half-time of folding of the order of $\sim 10^1$ to 10^2 ns (Williams *et al.*, 1996; Thompson *et al.*, 1997). This correlation is encouraging and suggests that the simulation of peptide folding in water is possible. Using the rate of unfolding from a single simulation to estimate how stable is a particular fold is unreliable. However, a significant change in the relative populations of the folded and unfolded conformations with increasing temperature was observed. Classifying all conformations with a backbone RMSD from the experimental structure of 0.10 nm for residues 2 to 6 to be folded, and all conformations with an RMSD greater than 0.15 nm to be unfolded, we can crudely estimate the relative free energy between the folded and unfolded conformations to be $\Delta G_{\text{folding}}(340\text{ K}) = 0.1$ kJ mol^{-1}, $\Delta G_{\text{folding}}(350\text{ K}) = 1.7$ kJ mol^{-1} and $\Delta G_{\text{folding}}(360\text{ K}) = 3.5$ kJ mol^{-1}. An uncertainty of a factor of 2 in the ratio of folded to unfolded conformations corresponds to an error of approximately 2 kJ mol^{-1} in $\Delta G_{\text{folding}}$ at any of these three temperatures.

The unfolded state

The unfolded state is most populated in the simulation at 360 K. By examining the RMSD between all structures extracted from the trajectory at 360 K every 0.01 ns (5000 structures) it is evident that the simulation does not randomly sample conformational space. Instead, the system hops between a series of discrete conformations. The system is in rapid equilibrium and detection of separate conformers at this temperature would not be possible using most experimental techniques. Two structures were considered equivalent or to have the same conformation if the backbone RMSD was less than 0.1 nm (residues 2 to 6). Of the 310 separate conformers identified by this criterion the system spends approximately 50% of the total time sampling just five. The predominant conformer is the 3_1-helix, which is present for approximately 25% of the total time. No other meta-stable fold incorporating the entire molecule that could be considered as a secondary structure has been identified. Of the 309 conformers representing the unfolded state, the system spends approximately 50% of the time in the unfolded state sampling just ten. The finding that the conformation of the peptide in the unfolded state is not random is in line with NMR studies on residual structure in denatured proteins (Dobson, 1992; Neri *et al.*, 1992; Dyson & Wright, 1993; Shortle, 1996) and has implications in regard to models for protein folding (Smith *et al.*, 1996).

Simplified force-fields for protein fold recognition and folding prediction

In regard to the development of simplified force-fields for protein fold recognition or protein folding prediction, it should be noted that none of the parameters or properties examined, the radius of gyration of the peptide, the polar or non-polar solvent-accessible surface area or various potential energy terms of the interaction function is, by itself, predictive of the folded state. Still, it is possible that, by assigning appropriate weights, a scoring function based on a combination of these parameters could be found to select the folded conformation. It is less likely, however, that such a scoring function or potential energy expression would predict the temperature-dependent distribution between folded and unfolded species in solution. The dependence of $\Delta G_{\text{folding}}$ on temperature suggests that folding is associated with changes in (solvent) entropy. If the solvent was water this would be the so-called hydrophobic effect. As a global property of a system, such entropy changes cannot easily be modelled by an implicit solvent. In fact, the basic principle of implicitly modelling entropic effects in terms of surface area or corrections to pairwise forces has been questioned (Mark & van Gunsteren, 1994; Dill, 1997). At the very least, the surface-area terms commonly used would need to be temperature-dependent. Simulations of peptides *in vacuo* are dominated by the multiple minima problem (Ripoll & Scheraga, 1988). This is evident in Figure 4A, which shows the intra-molecular potential energy as a function of the proximity to the folded conformation. Many configurations have an intra-molecular potential energy comparable to or lower than the folded conformation. The effect of inclusion of the explicit solvent is to smooth the potential energy surface. The simulations are computationally more expensive, but less likely to become trapped in local minima.

Conclusions

We believe we have, for the first time, demonstrated the possibility of simulating the reversible folding of a peptide in solution, albeit of a non-natural β-peptide, in atomic detail. We have demonstrated a shift in the equilibrium between folded and unfolded states as a function of temperature and have folded the peptide from a totally extended conformation at 340 K and 360 K. The explicit simulation of solvent molecules appears to be a prerequisite to correctly predict the folding/unfolding equilibrium as a function of temperature. At 360 K, a temperature exceeding the melting temperature of this peptide in the force-field, we have shown that the system rapidly hops between a relatively small number of states including the conformation stable at 298 K. The actual process of folding and unfolding was fast (sub-nanosecond) but occurred infrequently. In contrast to what is commonly assumed, the folding

and unfolding of peptides can occur on an accessible time-scale and current empirical force-fields can reproduce much of the available experimental data. Although the 21 rotatable dihedral angles of the peptide backbone span a conformational space of approximately 3^{21} ($\sim 10^{10}$) distinct conformers, only about 10^1 to 10^2 of these are significantly populated at 20 K above the melting temperature. The search problem in peptide folding is apparently surmountable. These results open a wide range of possibilities for the use of MD simulations in understanding the process of peptide folding and predicting possible folds of peptides in solution.

Methods

All simulations were performed using the GROMOS96 simulation program package in conjunction with the GROMOS force-field 43A1 (van Gunsteren *et al*., 1996). The β-amino acid residues were created based on their α-amino acid equivalent. The simulation at 298 K starting from the folded conformation (Figure 2A) contained the peptide and 962 methanol molecules in a rectangular periodic box, the dimensions of which were chosen such that the minimum distance from the peptide to the box wall was 1.4 nm in the starting configuration. The simulations at 340 K and 350 K were branched from the simulation at 298 K after 0.2 ns, the temperature being directly raised to 340 and 350 K, respectively. The simulations at 340 K and 360 K starting from the extended conformation (Figure 2B) contained the peptide and 1778 methanol molecules in a periodic truncated octahedron with again the minimum distance from the peptide to the box wall being 1.4 nm. The starting structures for the 14 simulated-annealing simulations corresponded to the seven conformations with largest radius of gyration selected from a 2.2 ns simulation at 400 K described previously (Daura *et al*., 1997). The temperature was lowered to 300 K using two different protocols. (i) By successively lowering the temperature by 20 K over five 0.05 ns intervals, and (ii) by successively lowering the temperature by 10 K over ten 0.025 ns intervals. The simulations were then continued for 0.5 ns at 300 K. All simulations were performed using periodic boundary conditions, at constant temperature and pressure, in conjunction with a twin-range cut-off for the non-bonded interactions of 0.8/1.4 nm. Unless stated otherwise, the parameter settings for the simulations were as described by Daura *et al*. (1997). Analysis of the trajectories was performed using programs from GROMOS96 (van Gunsteren *et al*., 1996) and SYMLIS (Krüger *et al*., 1991). Solvent-accessible surface areas were calculated using the program NACCESS (Hubbard & Thornton, 1993).

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