Molecular dynamics simulation of unfolding of histidine-containing phosphocarrier protein in water

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(Received : October 20, 1997 ; Accepted for publication : November 5, 1997 ; Published on Web : June 6, 1998)

Thermal unfolding of the histidine-containing phosphocarrier protein (HPr) was investigated by computation. Molecular dynamics simulations of HPr were performed in aqueous solution for 200 ps at 300 K and for 1 ns at 373 K. The initial structure remained stable during the simulation at 300 K; however, two major structural changes as the unfolding intermediates were observed during the simulation at 373 K.

Keywords: HPr, Protein unfolding, Molecular dynamics, Molten globule state, Transition state

1 Introduction

The theoretical study of the process of protein unfolding provides some insights into the mechanism of protein folding, which remains an important subject in molecular biology. Recently, it has been reported that several proteins can exist in partially structured states which are stable under certain conditions [1-5]. Such structures are known to be in 'molten globule states'. In a molten globule state, a structure appears to be compact, to be mobile and to retain secondary structure but not native tertiary structure [4]. Although the structural details of the native, folded conformation of proteins have been determined, those of the intermediates of folding/unfolding are difficult to characterize by experiments due to large fluctuations and the lack of a fixed structure throughout the molecules [6-10].
Molecular dynamics (MD) simulation is useful for understanding protein structures and dynamical properties. In principle, MD can reveal the details of the intermediate structures and dynamic transitions that occur during the unfolding process. For these reasons, we have performed high temperature MD simulation of protein unfolding. Histidine-containing phosphocarrier protein (HPr) was chosen because it is small (87 residues, Mr = 9315) and its crystal structure is known [11]. HPr consists of three α-helices (named A ~ C) and a four-stranded β-sheet (named b1 ~ b4) (Figure 1) [11].

Several MD simulations of temperature-induced protein unfolding have been reported. Fan et al. [12] performed high temperature (500 and 1000 K) vacuum MD simulation to study the molten globule state of α-lactalbumin. They found that constraining the helices does not stabilize the hydrophobic core, whereas constraining the hydrophobic core does stabilize the α-helices. Daggett & Levitt [13, 14] conducted high temperature MD simulations of the unfolding of bovine pancreatic trypsin inhibitor (BPTI). They performed five simulations: native BPTI at 298 K and 423 K and fully reduced BPTI at 298 K, 423 K, and 498 K. The reduced BPTI at high temperature indicated expansion of the protein. The authors concluded that the BPTI expansion was not caused by solvent penetration but by disruption of the packing interactions. Mark and van Gunsteren [15] performed MD simulations of the thermal denaturation of hen egg white lysozyme (500 K). They concluded that the lysozyme was still highly compact during the unfolding process, although the secondary structure was disrupted. MD simulations of apomyoglobin in water were performed by Brooks [16] at 312 K and by Tirado-Rives and Jorgensen [17] at 298K and at 358 K. In both simulations helices A, E, G and H were stable, while the other helices showed different behavior. This difference was due to the use of different destabilizing conditions. Caflisch and Karplus [18, 19] investigated the unfolding of barnase by high temperature MD simulations in the presence of explicit water molecules at 360 K and low pH, and at 600 K and neutral pH. They concluded that an essential step in the denaturation process is the separation of the major α-helix from the β-sheet, coupled to the exposure of the principal hydrophobic core, and that many non-polar side chains form hydrogen-bonds to water molecules. Li and Daggett [20] conducted MD simulations of the thermal unfolding of chymotrypsin inhibitor 2 (CI2) at 498K. They identified and characterized the major transition state of the unfolding process. They reported that in the transition state the molecule has a considerably weakened hydrophobic core, but that its overall structure is closer to that in the native rather than the unfolded state.

In most of the previous MD simulations, the unfolding process was accelerated by extremely high temperature (400 to 1000 K). HPr is a very small (87 residues) and simple protein containing three α-helices and a β-sheet. Hence, we expected that HPr would undergo unfolding without any of the additional acceleration conditions used in other MD simulations such as unreasonably high temperature or low pH [12–20]. To elucidate details of the unfolding pathway, we performed MD simulation of HPr for 1 ns at 373K.

2 Materials and Methods

2.1 Software for molecular dynamics

AMBER 3.0 Revision A [21] and a locally modified MD-module for the calculation of long-range electrostatic forces by twin-range methods (MD-MODIFIED) [22] were used throughout this study. The LINK, EDIT, and PARM modules were employed for construction of molecular
2. Generation of initial structure

The X-ray crystal structure of *Streptococcus faecalis* HPr [11] was used as the initial structure. An AMBER all atom force field [23] was employed during the simulations. The HPr structure was placed in a box filled with TIP3P water [24]. The box size (52 Å x 46 Å x 45 Å) was chosen so that the minimum distance of any protein atom from the wall of the box was 7.0 Å. Water molecules whose oxygens were located within 3.0 Å or whose hydrogens were located within 2.8 Å from the protein atoms were deleted. See Table 1 for other details of the simulation.

2.3 Molecular dynamics simulation

Two MD trajectories were generated, one at 300 K (room temperature simulation) and the other at 373 K (high temperature simulation). The energy of the initial structure was minimized until the norm of the gradient fell within 0.2 kcal/mol. In MD, all the covalent bonds were constrained to their equilibrium values by the SHAKE algorithm [25], and a time step of 2 fs was used. A twin-range method was employed to calculate nonbonded interactions [22, 26] where the first cut-off was 7Å and the second was 18 Å (see the legend to Table 1). Periodic boundary condition was applied to avoid the surface effect. The velocities were generated for the energy-minimized structure according to the Boltzmann distribution at 0.1 K. One hundred
steps of classical molecular dynamics with a time step of 0.1 fs and with velocity scaling every 10 steps were performed to prevent failure in SHAKE during the following heating period. Heating was done up to 300 K for 10 ps (room temperature simulation) or 373 K (high temperature simulation) for 25 ps. The temperature was kept constant at 300 K or 373 K according to Berendsen et al. [27] with a time constant of 0.2 ps. The simulations were continued at 300 K for a further 190 ps (total 200 ps) and at 373 K for a further 975 ps (total 1 ns). An NTP (constant number of particles, temperature, and pressure) ensemble at a pressure of 1 atm with a relaxation time of 0.5 ps was employed [27].

Table 1. Summary of simulation.

| Number of atoms       | 1307 (Protein) |
|-----------------------|----------------|
|                       | 7980 (Water)   |
|                       | 9287 (Total)   |
| Nonbonded cut-off radius (Å) | 7^a |
|                       | 18^b           |
| Number of nonbonded pairs | 8.2 × 10^5^a   |
|                       | 1.1 × 10^7^b   |
| Dielectric            | 1              |
| Box size (Å)          | X = 52         |
|                       | Y = 46         |
|                       | Z = 45         |
| CPU time usage        | 315 hours for 1000 ps |

^a^ For proximal pairs, van der Waals, H-bond and electrostatic forces were calculated for every timestep.

^b^ For long-range pairs, electrostatic forces were calculated once in 10 steps (immediately after up-date of the pairlists) and used in the following 9 steps.

^c^ Approximate values at 300 ps.

2.4 Analyses

The simulated structures were visualized by a graphic soft ware, Chemistry Viewer, on a Titan 1500 mini-super computer (Kubota Computer Co.). Analyses were performed using originally developed programs (References [22, 28] and this study).

3 Results and Discussion

3.1 Global structure

The root mean square deviation (RMSD) of the heavy atoms from the crystal structure as a function of time is given in Figure 2. The room temperature simulation was stable for 200 ps. In the high temperature simulation (373 K), two structural transitions were observed. Following
the initial transition (150 ~ 230 ps), the RMSD stabilized at approximately 7 Å between 230 to 400 ps (the first intermediate; FI). After 400 ps, the RMSD began to drift again (the second transition 400 ~ 840 ps). After 840 ps, the RMSD stabilized at approximately 12 Å (the second intermediate; SI). The deviation from the crystal structure observed during MD simulations was mostly distributed in the loop region of the structure, consistent with the other simulations (Figure 3) [13, 18].

3.2 Solvent-accessible surface area

Solvent-accessible surface area (ASA) was calculated according to Shrake and Rupley [29]. The radius of the probe solvent was 1.4 Å. Figure 5 shows the time course of the ASA during the simulations. At room temperature, the ASA was constant with an average value of 5187 Å² from 150 to 200ps (the ASA of the X-ray structure is 5162 Å²). At high temperature the ASA increased gradually until about 600 ps, and then stabilized at a value of about 8500 Å² (Figure 5)(the ASA of the fully extended structure is 14224 Å²). The ASA of the polar residues increased very slowly during the simulation (the ASA of the polar residues of the fully extended structure is 7813 Å²), but exposure of the nonpolar residues to the solvent occurred until about 400 ps (Figure 5)(the ASA of the nonpolar residues of the fully extended structure is 6411 Å²). The ASAs of the side-chains of polar residues are shown in Table 2. Most of the hydrophobic residues were exposed to the solvent in the FI state. These results suggested that the first
transition to FI caused the large RMSD and RMSF of the loop region of the structure (Figures 3, 4), along with the exposure of the nonpolar residues to the solvent.

### 3.3 Identification of the transition state

In the high temperature simulation, the first major structural change occurred around 150 ps (Figure 2). At 150 ps, A - C and b4 - C secondary structure element packings began to be disrupted (Figure 6). This resulted in opening of the hydrophobic core and exposure of the core residues to solvent. The unfolding of the hydrophobic core occurred very rapidly and most of the native hydrophobic contacts were lost during initial transition (150 ~ 230 ps) (Table 2 and Figure 5). Based on observations, the structure at 150 ps appeared similar to that in the native state, except for helix C (Figure 7). Helix C was separated from the hydrophobic core in this state. However, no solvent molecule penetrated between A and C, and b4 and C secondary structure elements. Energetically, loss of van der Waals interactions within the protein due to the disruption of the hydrophobic core packing was not compensated by protein - solvent interaction. Therefore, the enthalpy of the system should be high. Correspondingly, the potential energy of the protein calculated from the coordinates at this state increased. At 150 ps, the protein was still relatively compact (Figures 5-7), and the entropy was surmised to be a little higher than that in the native state. Thus, the free energy at this time point should be high, and consequently, the unfolding of the protein occurred quickly (Figure 2). From these considerations, we think the structure at 150 ps to be close to that in the transition state, which may be a transient intermediate. Li & Daggett reported similar characteristics in the transition state of CI2 unfolding [20].
Figure 4. Main chain RMS fluctuations of the residues in HPr. The continuous thick line shows the RMS fluctuations calculated from the X-ray temperature factors [11]. The dotted thick line shows the RMS fluctuations calculated from the average values over the 150 ~ 200 ps period of the room temperature simulation. The thin continuous line and thin dotted line show the RMS fluctuations calculated from the average values over the 250 ~ 350 ps and 900 ~ 1000 ps period of the high temperature simulation, respectively.

Figure 5. Solvent-accessible surface area (ASA) as a function of time. The continuous thick line shows ASA of the whole protein at high temperature. The continuous thin line shows ASA of the whole protein at room temperature. The dashed thick line shows ASA of hydrophobic residues in HPr, and the dotted thick line shows ASA of polar residues in HPr at high temperature.
Table 2. Side chain ASA at 373 K.

| Residues | X-ray structure | 250 \sim 350 \text{ ps}^a | 900 \sim 1000 \text{ ps}^a |
|----------|-----------------|----------------------------|-----------------------------|
| Phe 6    | 48.5            | 101.6                      | 168.5                       |
| Ile 8    | 0.0             | 117.9                      | 141.4                       |
| Ile14    | 0.0             | 111.5                      | 121.0                       |
| Val23    | 0.0             | 70.7                       | 108.5                       |
| Ala26    | 0.0             | 22.7                       | 64.5                        |
| Phe29    | 20.8            | 21.4                       | 106.1                       |
| Leu35    | 0.0             | 17.3                       | 86.7                        |
| Leu44    | 0.0             | 10.8                       | 133.5                       |
| Val55    | 0.0             | 105.0                      | 64.9                        |
| Val61    | 0.9             | 100.5                      | 111.9                       |
| Ile63    | 0.0             | 30.0                       | 110.0                       |
| Val65    | 0.0             | 32.6                       | 103.4                       |
| Ile77    | 0.0             | 45.6                       | 120.2                       |
| Leu86    | 0.0             | 108.7                      | 218.6                       |
| Ala87    | 53.4            | 176.6                      | 120.5                       |
| Total$^b$| 5162            | 6860                       | 8514                        |

$^a$ ASA time-averaged over a 100 ps period.

$^b$ The total ASA of HPr including polar and nonpolar residues.

Figure 6. Interatomic Cα-Cα distances as a function of time at 373K. Continuous line, the distance between Ala26 Cα and Ile77 Cα atoms; broken line, the distance between Ile63 Cα and Ile77 Cα atoms. The 26-77 and 63-77 distances refer to A-C and b4-C hydrophobic packing, respectively.
3.4 Secondary structure

Secondary structure was assigned based on the backbone dihedral angles, φ and ψ. The residues are considered to reside in the helical region of the conformational space when φ and ψ angles are within approximately 40° of the most commonly observed values in the helical regions of crystal structures (-100° ≤ φ ≤ -30°; -80° ≤ ψ ≤ -5°, where the canonical values are -63.8° ± 6.6° and -41.0° ± 7.2° for φ and ψ, respectively [30]). The β-region was bounded as follows: -170° ≤ φ ≤ -50°, 80° ≤ ψ ≤ 190°. The secondary structure content of the simulated structure at high temperature was calculated with the requirement that at least three successive residues fulfill the angular criterion as described above at any one time.

The α-helices were stable during this simulation (percentage of α-helices within the HPr was about 40 %; Figure 8). In contrast to α-helices, the β-sheet content began to decrease at about 300 ps, and completely disappeared after 800 ps (Figure 8). These results indicated that FI retained most of its secondary structure, but SI retained only α-helices. In the case of α-lactalbumin, most α-helix regions were stable while β-sheet regions were unfolded in the molten globule state, as revealed by NMR and CD spectroscopy [7, 31]. This suggests that SI of HPr is in a molten globule state.

3.5 Packing interactions

Figure 9 shows the residue-residue contacts present in the HPr crystal structure (upper-left diagonal of Figure 9 (A), (B) and (C)). in the averaged structure in the last 50 ps of the room temperature simulation (lower-right diagonal of Figure 9 (A)), in the averaged structure from 250 to 350 ps in the high temperature simulation (FI state; lower-right diagonal of Figure 9 (B)), and in the averaged structure from 900 to 1000 ps in the high temperature simulation (SI state; lower-right diagonal of Figure 9 (C)).

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In the crystal structure, various tertiary contacts are evident (see detail for Table 3), nearly all of which were maintained in the room temperature simulation (Figure 9 (A)). These tertiary contacts were also observed at the high temperature simulation between 250 and 350 ps (FI state; Figure 9 (B) and Table 3). In contrast, most of the tertiary contacts were lost at high temperature between 900 and 1000 ps (SI state; Figure 9 (C) and Table 3). In FI state several $\beta$-$\beta$, $\alpha$-$\beta$ and $\beta$-$\beta$ contacts were observed, but in the SI state only an $\alpha$-$\alpha$ contact (A - B) was observed (Table 3), because the $\beta$-sheets had been disrupted. These results again indicate that SI exists in a molten globule state.

4 Conclusion

We performed MD simulations of HPr to analyze the protein unfolding pathway. The simulation at room temperature was stable, but the protein underwent two-step unfolding at high temperature. Both the first and second intermediates fluctuated greatly. In particular, the loop regions deviated markedly from the crystal structure and showed large fluctuations, consistent with other high temperature simulations [13, 18]. Most of the hydrophobic residues were exposed to the solvent during the first structural transition. All of the secondary structures were maintained in the first intermediate between the first and second transitions, but in the second intermediate, the $\beta$-sheet was completely lost although $\alpha$-helices were stable. The tertiary contacts were fairly well maintained in the first intermediate; in contrast, those in the second intermediate were diminished.

The structure during the transition state of unfolding was identified from the high temperature MD simulation. The overall structure during the transition state was closer to that in the native than the unfolded state. The disruption of the hydrophobic packing of A - C and
Figure 9. Tertiary contact maps. Crystal structure (upper-left diagonal of (A), (B) and (C)), room temperature simulation (lower-right diagonal of (A)), high temperature simulation during 250 to 350 ps (lower-right diagonal of (B)) and 900 to 1000 ps (lower-right diagonal of (C)). The darkest blocks represent Cα-Cα distances $\leq 5$ Å, the second lightest shade is for $5 \text{ Å} \leq d \leq 7$ Å, and the lightest shade is for $7 \text{ Å} \leq d \leq 10$ Å.
b4 - C, without solvent penetration was the first step of structural change from the native to transition state. The transition state occurred early in the unfolding process, and involved a transient intermediate (which quickly unfolded to another state).

To date no experimental data are available on the thermal unfolding of HPr. However, the unfolding of HPr based on our simulation was considered to involve the following; (1) the disruption of hydrophobic packing, but no penetration to the inside of the opened secondary structure elements by water molecules (transition state), (2) increase of the mobility of the loop region and simultaneous exposure of the hydrophobic residues to the solvent (FI), and (3) cooperative disruption of the β-sheet and tertiary packing (SI) (Figure 10). We considered SI to be in a molten globule state because it showed large fluctuations and a considerable amount of the native secondary structure was preserved while the tertiary structure was completely destroyed.

### Table 3. Percentage of tertiary contact.

| contact (Native) | 250 ~ 350 ps<sup>b</sup> | 900 ~ 1000 ps<sup>b</sup> |
|-----------------|-----------------|-----------------|
| b1 - b4         | ○               | ×               |
| b1 - C          | ×               | ×               |
| b1 - b2         | ○               | ×               |
| A - B           | △               | ○               |
| A - C           | ×               | ×               |
| A - b2          | △               | ×               |
| b2 - b3         | ○               | ×               |
| b2 - B          | ○               | ×               |
| b2 - b4         | ×               | ×               |
| b3 - B          | ○               | ×               |
| b3 - b4         | ×               | ×               |
| b4 - C          | ×               | ×               |

<sup>a</sup> The secondary structure segments are shown in Figure 1.

<sup>b</sup> Percentage time-averaged over a 100 ps period.

○: 80 ≤ %, ○: 50 ≤ % ≤ 80, △: 30 ≤ % ≤ 50, ×: % ≤ 30
We thank RIPS at the Agency of Industrial Science and Technology for providing time on the Cray C916 supercomputer. This work was partly supported by a research grant from the Sumitomo Foundation (to I. Y.) and by the RING Program of the Agency of the Industrial Science and Technology (to Y. K. and M. U.).

References

[1] Dolgikh, D.A., Gilmanshin, R. I., Brazhnikov, E. V., Bychkova, V. E., Semisotnov, G. V., Venyaminov, S. Y. and Ptitsyn, O. B., FEBS Letters, 136, 311-315 (1981).

[2] Ptitsyn, O. B., Pain, R. H., Semisotnov, G. V., Zerovnik, E. and Razgulyaev, O. I., FEBS Letters, 262, 20-24 (1990).

[3] Kuwajima, K., Proteins, 6, 87-103 (1989).

[4] Ohgushi, M. and Wada, A., FEBS Letters, 614, 21-24 (1983).

[5] Ptitsyn, O. B. “The molten globule state.” in Protein folding. (Creighton, T. E. eds.) 243-300 (1992) W. H. Freeman, New York.

[6] Amir, D. and Haas, E., Biochemistry, 27, 8889-8893 (1988).

Figure 10. Main chain trace of the averaged structures in the high temperature simulation of HPr. See legend to Figure 1 for colors of the secondary structures.
[7] Baum, J., Dobson, C. M., Evans P. A. and Hanley, C., *Biochemistry*, 28, 7-13 (1989).

[8] Dobson, C. M., *Curr. Opin. Struct. Biol.*, 201, 161-200 (1992).

[9] Harding, M. M., Williams, D. H. and Woolfson, D. N., *Biochemistry*, 31, 3120-3128 (1991).

[10] Hughson, F., Wright, P. and Baldwin, R. L., *Science*, 249, 1544-1548 (1990).

[11] Jia, Z., Vandonselaar, M., Hengstenberg, W., Quail, J. W. and Delbaere L. T. J., *J. Mol. Biol.*, 236, 1341-1355 (1994).

[12] Fan, P., Kominos, D., Kitchen, D. B., Levy, R. M. and Baum, J., *Chemical Physics*, 158, 295-301 (1991).

[13] Daggett, V. and Levitt, M., *Proc. Natl. Acad. Sci. USA*, 89, 5142-5146 (1992).

[14] Daggett, V. and Levitt, M., *J. Mol. Biol.*, 232, 600-619 (1993).

[15] Mark, A. E. and van Gunsteren, W. F., *Biochemistry*, 31, 7745-7748 (1992).

[16] Brooks, C. L., *J. Mol. Biol.*, 227, 375-380 (1992).

[17] Tirado-Rives, J. and Jorgensen, W. L., *Biochemistry*, 32, 4175-4184 (1993).

[18] Caflisch, A. and Karplus, M., *Proc. Natl. Acad. Sci. USA*, 91, 1746-1750 (1994).

[19] Caflisch, A. and Karplus, M., *J. Mol. Biol.*, 252, 672-708 (1995).

[20] Li, A. and Daggett, V., *J. Mol. Biol.*, 257, 412-429 (1996).

[21] Seibel, G., Singh, U. C., Weiner, P. K., Caldwell, J. and Kollman, P. A., *AMBER 3.0 Revision A*, University of California, San Francisco, CA. (1989).

[22] Komeiji, Y., Uebayasi, M., Someya, J. and Yamato, *Prot. Engng*, 4, 871-875 (1991).

[23] Weiner, S. J., Kollman, P. A., Nguyen, D. and Case, D. A., *J. Compt. Chem.*, 7, 230-252 (1986).

[24] Jorgensen, W. L., Chandrasekhar, J., Madura, J. D., Impey, R. W. and Klein, M. L., *J. Chem. Phys.*, 79, 926-935 (1983).

[25] Ryckaert, J., Ciccotti, G. and Berendsen, H. J. C., *J. Comput. Phys.*, 23, 327-341 (1977).

[26] De Vlieg, J., Berendsen, H. J. C. and van Gunsteren, W. F., *Proteins*, 6, 104-127 (1989).

[27] Berendsen, H. J. C., Postma, J. P. M., van Gunsteren, W. F., DiNola, A. and Haak, J. R., *J. Chem. Phys.*, 81, 3684-3690 (1984).

[28] Komeiji Y., Uebayasi M. and Yamato, *Proteins*, 20, 248-258 (1994).

[29] Shrake, A. and Rupley, J. A., *J. Mol. Biol.*, 79, 351-371 (1973).

[30] Presta, L. G. and Rose, G. D., *Science*, 240, 1632-2682 (1988).
[31] Chyan, C.-L., Wormald, C., Dobson, C. M., Evans, P. A. and Baum, J., Biochemistry, 32, 5681-5691 (1993).
ヒスチジン含有リン酸転移タンパク質変性過程の分子動力学シミュレーション

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ヒスチジン含有リン酸転移タンパク質 (HPr) 熱変性過程の分子動力学シミュレーションを、水中で行った。温度300Kで200ピコ秒、373Kで1ナノ秒シミュレーションした結果、300Kのシミュレーションでは初期構造は安定であった。これに対し373Kでは、シミュレーションの間に2つの主な構造変化が見られた。

キーワード: HPr, Protein unfolding, Molecular dynamics, Molten globule state, Transition state