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Dynamical properties of bovine pancreatic trypsin inhibitor from a molecular dynamics simulation at 5000 atm

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Molecular dynamics simulations of bovine pancreatic trypsin inhibitor in water have been performed with coupling to pressure baths at 1 atm and at 5000 atm. The positional fluctuations of atoms in the α- and γ-positions are slightly decreased at 5000 atm. The mobility of the backbone ϕ- and ψ-angles is not affected with respect to the root mean square fluctuations and the rate of torsional angle transitions. The amplitude of libration of sidechain χ-angles remains nearly the same for both pressures, but the rate of torsional angle transitions decreases on average by 30% when increasing the hydrostatic pressure to 5000 atm.

BPTI; Molecular dynamics; Hydrostatic pressure; Computer simulation

1. INTRODUCTION

Experimental data on the structural and dynamical behaviour of proteins at high pressure is becoming more available. Williams et al. [1] studied the denaturation of poly[N^5-(3-hydroxypropyl)-L-glutamine] by NMR-spectroscopy at pressures up to 1905 atm. Wagner [2,3] determined the effect of pressures up to 1185 atm on the ring flip rates of tyrosine and phenylalanine in BPTI by NMR spectroscopy. A theoretical interpretation of Wagner's results was given by Karplus and McCammon [4]. These ring flip processes are much too slow to be studied by the molecular dynamics (MD) method, yet. A more detailed structural analysis of a protein with respect to high pressure has been provided by Kundrot and Richards [5]. In an X-ray diffraction study of hen egg-white lysozyme at 1000 atm they found on average a decrease in the atomic B-factors of 0.01 nm^2, slightly deformed secondary structure regions and an expanded loop (residues 61 to 87). A computer simulation study of a lysozyme crystal unit cell including water would require prohibitively large computing resources. Therefore, we studied the effects of high pressure on the structure and fast motions of a smaller, well-characterized model peptide, BPTI [6–11] by MD simulation.

Two simulations at pressures of 1 atm and 5000 atm and at T = 277K are compared. Recently, Kitchen et al. [12] performed a similar study, but at different temperature (T = 298K) and pressure (P = 1 bar and P = 10 kbar). Although the molecular model, atomic interaction function, simulation set-up, etc. of [12] differ from the ones used here, the structural results are comparable. The differences can be explained on the basis of the differences in temperature and pressure. Where Kitchen et al. [12] give an extensive account of structural and energetical data, we present a short summary of structural and dynamical results.

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Abbreviations: BPTI, bovine pancreatic trypsin inhibitor; MD, molecular dynamics; NMR, nuclear magnetic resonance; RMS, root mean square.

2. MATERIALS AND METHODS

The molecular dynamics simulations of BPTI in water were performed using the GROMOS force field [13] Non-polar hydrogen atoms were included in the carbon atoms (united atom approach), and polar hydrogen atoms were treated explicitly, resulting in 568 BPTI atoms. We used charged residues Arg^+, Lys^+, Glu^- and Asp^- resulting in a total charge of +6 e for BPTI. For the water molecules the SPC/E model was used [14]. The starting structure was obtained by a distance geometry calculation based on NMR data [11] and four internal water molecules were added. The resulting structure was energy minimized and placed in an truncated octahedron with 2371 SPC/E water molecules.

During the initial part of the equilibration of the BPTI/water system at a pressure of 1 atm the BPTI atoms were harmonically restrained to their start-up positions and the temperature was increased in two stages from 100K to 277K, the final simulation temperature. The details of the equilibration conditions are given in [15] and (Brunne et al., manuscript in preparation). The temperature of the protein and of the solvent were separately coupled to a temperature bath [16] using a coupling constant of 0.1 ps. The pressure was kept constant by coupling to a pressure bath at either 1 atm or 5000 atm [16] with a coupling constant of 0.5 ps. An isothermal compressibility of 45.63×10^{-6} atm^{-1} for water at 1 atm [17] was applied throughout. Bonds were always kept rigid using the SHAKE method [18] with a relative tolerance of 10^{-4}. A cut-off radius of 0.8 nm for the non-bonded interactions and of 1.2 nm for the long-ranged charged inter-

actions was used. The latter were only calculated every 10 steps, when the pair list was updated. The time step was 0.002 ps in all runs. For analysis the coordinates were stored every 0.1 ps.

The simulation at 1 atm stabilized after 400 ps in terms of C^{α}- and all atom RMS displacements with respect to the starting structure. During the simulation period of 400 to 500 ps the backbone dihedral angles did not undergo any conformational transitions and we used this period (A) to characterize the mobility of the dihedral angles and the atom positions. Coordinates and velocities after 400 ps were also used as starting point for the simulation at 5000 atm. Fifty picoseconds were allowed for equilibration and then the simulation was carried on for 200 ps, the analysis period. Because a conformational transition was observed for the ^{6}Leu/^{7}Glu peptide plane after ca. 110 ps we analyzed the first 100 ps (B) and the last 80 ps (C) separately, thus excluding effects that arise from conformational changes in the backbone of the protein.

3. RESULTS AND DISCUSSION

Table I lists various properties averaged from the three trajectories, A, B and C. The volume of the simulation box is approximately 12% smaller in the high pressure runs B and C. The number-densities, ρ_N, of the BPTI molecule, determined within a radius of 0.8 nm around the center-of-mass coordinates, increases by ca. 5%. This is in agreement with the slight decrease of the radius of gyration, r_G.

All three average conformations, A, B and C have an average RMS displacement for C^{α}-atoms of ca. 0.16 nm and for all atoms of ca. 0.26 nm relative to the X-ray structure [8]. The C^{α}-atoms of the loop regions of residues 10–15 and 25–28 deviate in conformations B (○) and C (□) of the high pressure simulation more from the X-ray structure than conformation A (●) of the normal pressure simulation, whereas the β-sheet regions 21–24 and 29–32 of conformations B and C are closer to the X-ray structure than those of conformation A (Fig. 1). The RMS fluctuation of atoms in the α- and γ-positions about their average positions $<(\Delta r_{\alpha,\gamma})^2>^{1/2}$ averaged over all residues is smaller in the high pressure simulations B (○) and C (□). The small effect of increasing the pressure on the atomic positional fluctuations is in agreement with the experimental results for crystalline lysozyme [5]. From Fig. 2 it can be seen that the lower average mobility is not due to a large decrease of the mobility in a few domains but that the mobility is slightly reduced throughout. Large, but considerably reduced mobility is still observed in the flexible loop regions around residues 16 and 26.

A significant effect upon the dihedral angle fluctuations, σ, which could be expected from a reduced mobility of the atoms, is not observed. The RMS fluctuations of the backbone dihedral angles remain essentially the same. The decrease in the average angle fluctuations is due to a slight decrease in the sidechain angle mobility as can be seen from the change in $\sigma(\chi_2)$, taken as representative for sidechain angles.

When comparing our structural results with those of Kitchen et al. [12], a very similar picture emerges, be it

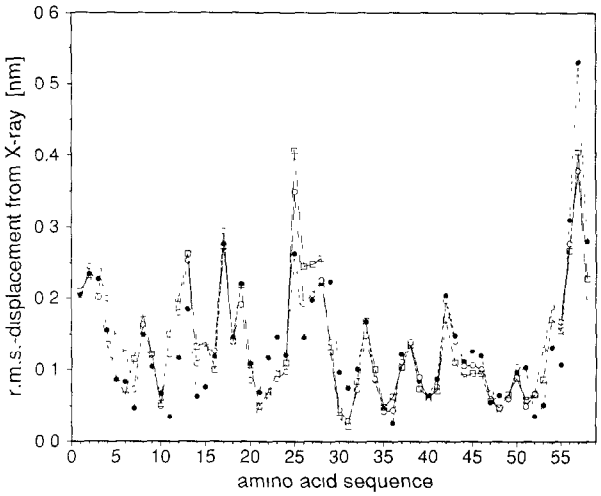

Fig. 1. RMS displacement of C^{α}-atoms of BPTI relative to the X-ray structure [8] as a function of the amino acid sequence. The displacements are determined from average structures of trajectory A (●), 100 ps at 1 atm, and of trajectories B (○), 100 ps at 5000 atm, and C (□), 80 ps at 5000 atm.

Table I

Properties of the conformations of BPTI averaged from a 100 ps MD simulation at 1 atm pressure (A), from the initial 100 ps of a simulation at 5000 atm (B), and from the final 80 ps at 5000 atm (C). 1 atm = 1.0325 bar

		A	B	C
Reference pressure	(atm)	1	5000	5000
Equilibration period	(ps)	400	400 + 50	400 + 170
Analysis period	(ps)	100	100	80
Box-volume	(nm^3)	76.2	66.8	66.7
Number density	(nm^{-3})	62.5	66.2	65.7
r_G	(nm)	1.15	1.14	1.14
$<(\Delta r_{\alpha})^2>^{1/2}$	(nm)	0.049	0.046	0.042
$<(\Delta r_{\gamma})^2>^{1/2}$	(nm)	0.066	0.056	0.052
$\sigma(\phi)$	(°)	14	14	15
$\sigma(\psi)$	(°)	13	13	13
$\sigma(\chi_2)$	(°)	28	25	20
σ(all)	(°)	18	17	15
$\nu_{da}(\phi)$	(ps^{-1})	1.13	0.98	1.25
$\nu_{da}(\psi)$	(ps^{-1})	0.92	0.84	0.91
$\nu_{da}(\chi_2)$	(ps^{-1})	1.23	0.57	0.75
$\nu_{da}(\chi_n)$	(ps^{-1})	3.31	2.46	2.23
ν_{da}(all)	(ps^{-1})	5.36	4.28	4.39

r_G, radius of gyration; $<(\Delta r)^2>^{1/2}$: positional RMS fluctuation of atoms in α- and γ-positions; σ, RMS fluctuation of ϕ-, ψ-, χ_2- and all dihedral angles, ν_{da}, transition rates of ϕ-, ψ-, χ_2-, all (except Pro) χ_n- and all dihedral angles.

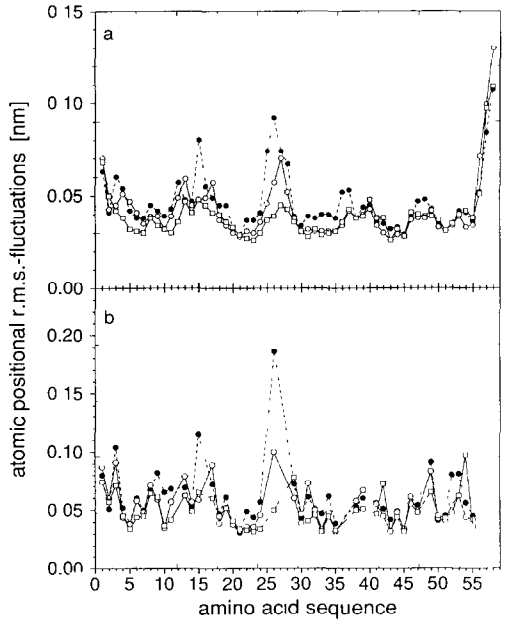

Fig. 2. Atomic positional RMS fluctuations of atoms in α- (a) and in γ- (b) positions as a function of the amino acid sequence of BPTI. The fluctuations are determined from trajectory A (●), 100 ps at 1 atm, and from trajectories B (○), 100 ps, and C (□), 80 ps at 5000 atm

that in [12] the pressure effects are more pronounced due to a twice as high pressure and a 21K higher temperature. This indicates that the differences in atomic model, force field, simulation set-up, et. between these two studies play a minor role.

We also studied dynamical effects of the pressure change. In addition to the dihedral angle fluctuations the number of dihedral angle transitions per time, ν_{da} was determined. A transition is counted as such whenever a dihedral angle crosses a barrier in the dihedral angle potential energy function and passes the bottom of the adjacent well. Transitions according to this definition can be accomplished by a change in the dihedral angle value of as little as 30°. Therefore, back and forth transitions over the same barrier need not to imply a conformational change. The rate of transitions of the backbone angles ϕ and ψ does not change upon an increase in pressure. The transition rates for all rotating χ-angles is about 30% smaller at 5000 atm, for the χ_2-angles about 50% smaller, than at 1 atm. Although the number of sidechain angle transitions decreases very much, the RMS fluctuations remain almost constant, because the librational amplitude for the angles remains nearly the same.

The small decrease in the atom position fluctuations and the considerable decrease in the transition rate of sidechain angles, while there is hardly any effect upon the backbone angles, with increase in pressure can be explained by a more dense packing of the protein and a higher density of the surrounding water, that especially increases the friction for sidechain motions.

The large decrease of the mobility in the more flexible loop regions when increasing the pressure indicates that the mobility of peptides that are less rigid than BPTI, is also expected to be lower, especially for their sidechain atoms. As more data from high pressure experiments become available it will also become important to test currently used force fields with respect to their applicability to high pressure simulations.

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