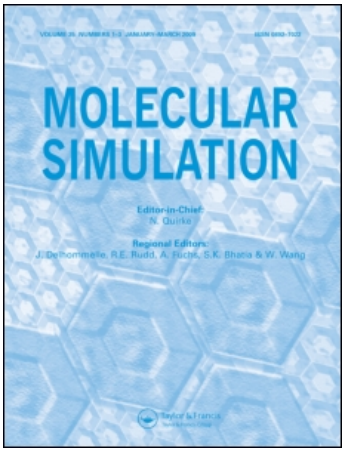

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Pressure and Temperature Dependence Study of the Denaturation of Ribonuclease A Solutions

Jeong Yun Yu[a]; Nam Sook Kang[a]; Mu Shik Jhon[a]
[a] Center for Molecular Science and Department of Chemistry, Korea Advanced Institute of Science and Technology, Taejon, Korea

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PRESSURE AND TEMPERATURE DEPENDENCE STUDY OF THE DENATURATION OF RIBONUCLEASE A SOLUTIONS

JEONG YUN YU, NAM SOOK KANG and MU SHIK JHON

Center for Molecular Science and Department of Chemistry,
Korea Advanced Institute of Science and Technology,
373-1, Kusung-dong, Yusung-gu, Taejon 305–701, Korea

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We have carried out molecular dynamics simulations on the Ribonuclease A solution to see the structural and energetic effects of the temperature and pressure on the protein denaturation and to compare the two effects. The simulations are done at 350 K for the thermal denaturation and at 6000 bar for the pressure denaturation. We used the consistent valence force field (CVFF) potential and the single point charge (SPC) water potential function to represent the interactions in the simulational systems. During 100 ps, both structures denatured by the temperature and pressure changes have retained the native-like framework. We have reported the structural changes of Ribonuclease A and water molecules through the root mean square displacement, the solvent accessible surface area including the contribution of each amino acid to the total solvent accessible surface area, and the hydrogen-bond pattern. We have examined the structure of water molecules in the systems by the distribution of small water clusters connected by the hydrogen-bonded circular network. The hydrophobic residues in the protein surface are more exposed by the thermal denaturation than by the pressure denaturation.

KEY WORDS: Pressure effect, temperature effect, protein conformation, molecular dynamics simulation, ribonuclease A.

1. INTRODUCTION

There are many factors that influence protein denaturation, i.e., pH, temperature, pressure, and solvent composition. Temperature, pressure and a combination of both are useful tools in the study of the mechanism of many biological processes containing protein folding. The value of pressure as a variable on the structural thermodynamics of proteins was mentioned in reference [1] where the authors pointed out that pressure provides the means for perturbing the environment of a molecule in a continuous and controlled way. However, the study of the effect of pressure upon proteins has been much less frequent than that of temperature [2]. The difficulty with pressure studies is fact that protein function and structure are strongly influenced by other environmental factors such temperature, pH, salt, and ligand binding (Michels and Clark [3]). Thus, much care must be taken when collecting data from pressure studies under different experimental conditions.

Most studies of the pressure and temperature effects have used spectroscopic tools [4–11] which cannot give detailed and direct information at the atomic level over the

entire structure (except chromophores) or have an experimental limitation in the pressure and temperature ranges. These shortcomings are compensated by computer simulation methods such as molecular dynamics (MD) simulations which can easily control the conditions and give a variety of analyses at the atomic level. A few studies on the protein denaturation by computer simulation are found in the literature [2, 12, 13].

Generally, the pressure denaturation of globular protein is reversible in the range of 4–8 kbar. Over this range, precipitations or gels can be formed. The denatured protein structure is not always like a random-coil, but may retain significant structures of the native proteins [5, 9]. The volume change during the pressure denaturation is small compared with the thermal denaturation. Protein stability is decided by many factors, *i.e.*, its structural characteristics, intramolecular interaction and environmental interaction. Among them, in particular, the hydrogen bond, electrostatic interaction, and hydrophobic effect have been treated as main subjects.

Ribonuclease A, a small monomeric enzyme, consists of 124 amino acids lacking tryptophan with an N-terminal α-helix and two shorter helices packed against a central, twisted antiparallel β-sheet [14]. Ribonuclease A from bovine pancreas has been the subject of many protein folding and stability studies over the years with lysozyme, chymotrysinogen, etc.

Water molecules connected by hydrogen bonds form various kinds of small clusters. Interest has been focused on the hydrogen-bonded circular networking. The ring distribution typically peaks at six, and it can be changed by its environment or condition such as temperature and pressure. In this study, we calculated the distribution of rings to investigate the change of water structure surrounding the Ribonuclease A with pressure and temperature denaturation, that is, by exposure of inner residues or hidding of surface residues during the denaturation.

In the present article, we performed molecular dynamics simulations on Ribonuclease A solutions to investigate the pressure and temperature effects on the protein denaturation and to compare the two effects. As analysis on the denatured proteins, we calculated the solvent accessible surface area and the contribution of each amino acid to the solvent accessible surface area. The change of water structure due to the rearrangement of surface residues by protein denaturation is detected by the distribution of water clusters connected by the hydrogen-bonded circular network.

2. METHODS

We performed molecular dynamics simulations for Ribonuclease A solutions (i) at 300 K and 6000 bar, (ii) at 350 K. The pressure and temperature were selected by considering the experimental results [6, 8]. The initial coordinates of Ribonuclease A were from the Brookhaven Protein Data Bank. First, all the hydrogen atoms in the protein were explicitly added using the Insight programme installed on Silicon Graphics. We placed the protein at the centre of the rectangular box of dimensions $x = 48.3$ Å, $y = 42.2$ Å, $z = 60.1$ Å and filled the space of the box with water molecules, resulting in the addition of 3343 water molecules and the system density of 1.002 g/cm^3.

The solvent molecules only were minimized by the steepest descent algorithm, fixing the protein, followed by full minimization for 5000 steps for the protein and the solvent

molecules. Molecular dynamics simulations for initializing the systems were then continued for 2000 steps with a time step of 1 fs. After that, runs were continued for 100 ps. The periodic boundary conditions were applied during the simulations. The equations of motion were integrated using a leapfrog algorithm which has been found to be the most stable of the commonly used algorithms in molecular dynamics, especially in NPT simulation [15]. The temperature and pressure of the systems were controlled by the Berendsen's method which simulates the coupling of the variables to an external bath [16]. The relaxation times for the temperature and the pressure scaling were 0.01 ps and 0.1 ps, respectively. We used the consistent valence force field (CVFF) potential developed by Hagler *et al.* [17] and single point charge (SPC) water potential function [18] to represent the interactions in the simulational systems with the cut-off distance of 10.0 Å. Also, the switching function was used to reduce smoothly the potential energy and to avoid discontinuities and energies. A non-bonded pair list which was used to accelerate calculations was updated every 25 steps.

The energetic criterion was used to define the hydrogen bond between water molecules in the analysis of the ring distribution and the criterion is an interaction energy of -2.50 kcal/mol or less. Its value nearly corresponds to the minimum of the pair-energy distribution of potential. Detailed explanations for selecting the kind of ring are given in the article of Yu *et al.* [19]. Solvent accessible surface area was calculated by Richard's method [20] with the probe radius of 1.4 Å.

3. RESULTS AND DISCUSSION

The structures of Ribonuclease A obtained by our simulations can be regarded as intermediates during the denaturation. They retain the framework of the native protein. Here, we analyzed them and compared the temperature denatured structure (*T*-denatured) with the pressure denatured one (*P*-denatured).

Table 1 represents the averages of various thermodynamics properties for the simulational systems. The system energies were converged within 4 ps. We obtained the average values from the last 60 ps trajectories during which the systems were very stabilized with respect to both energies and motions. Among potential energies, we reported the Coulomb and Lennard-Jones energies. Even though the *P*-denatured system is more stabilized than the *T*-denatured system, the Lennard-Jones energy of

Table 1 Thermodynamic results of simulations

	P-denatured (NPT)	*T-denatured* (NVT)
Total Energy (Kcal/mol)	−19827.72	−13962.99
Potential Energy (Kcal/mol)	−30454.92	−26362.82
Lennard-Jones Energy (Kcal/mol)	7053.25	5279.16
Coulomb Energy (Kcal/Mol)	−45664.94	−40255.11
Temperature (K)	299.98	350.01
Pressure (bar)	5999.75	
Volume (Å^3)	101272.87	122441.94
Density (g/cm^3)	1.212	1.002

P-denatured structure is higher than that of the *T*-denatured, which is due to the increase of non-bonded repulsion by compression. The density of the system by *P*-denaturation increases by 21% (1.212 g/cm^3) compared with its initial value (1.002 g/cm^3). As known by the compressibilities of globular proteins and water molecules [22], this increase is mainly due to the compression of solvent water molecules.

Figures 1 and 2 show the root mean square displacement (RMS) as a function of the number of MD steps for the protein atoms and the water molecules, respectively. The *P*-denatured system converged very fast and the fluctuation is small compared with the *T*-denatured system. For each system, the values of side chains are larger than that of backbone atoms and the trend of the curves is very similar to that of Figure 1, even though the curves are not reported here. Water molecules around *T*-denatured protein move very fast compared with those around *P*-denatured one, which can be easily expected from the temperature of the systems.

We calculated the number of hydrogen bonds existing in the systems minimized after 102 ps MD and reported the ratio of the intra-protein hydrogen bond and the protein-water hydrogen bond to the total hydrogen bond in Table 2, with the ratio in the initial minimized system. There is no noticeable difference in the ratio though the absolute number of the hydrogen bond of *P*-denatured system is much larger than that

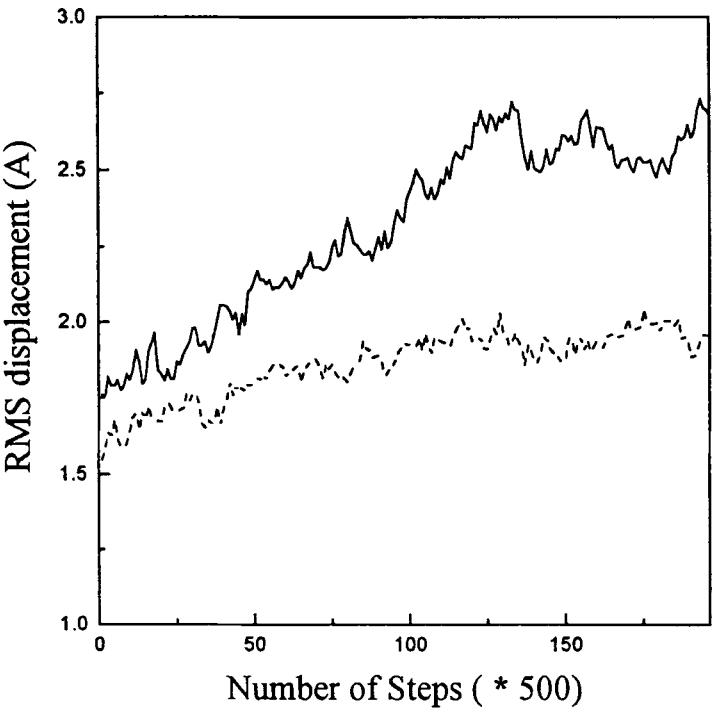

Figure 1 The root mean square displacement (RMS) of protein atoms as a function of a number of MD steps. The solid line represents the *T*-denatured system and the dashed line the *P*-denatured.

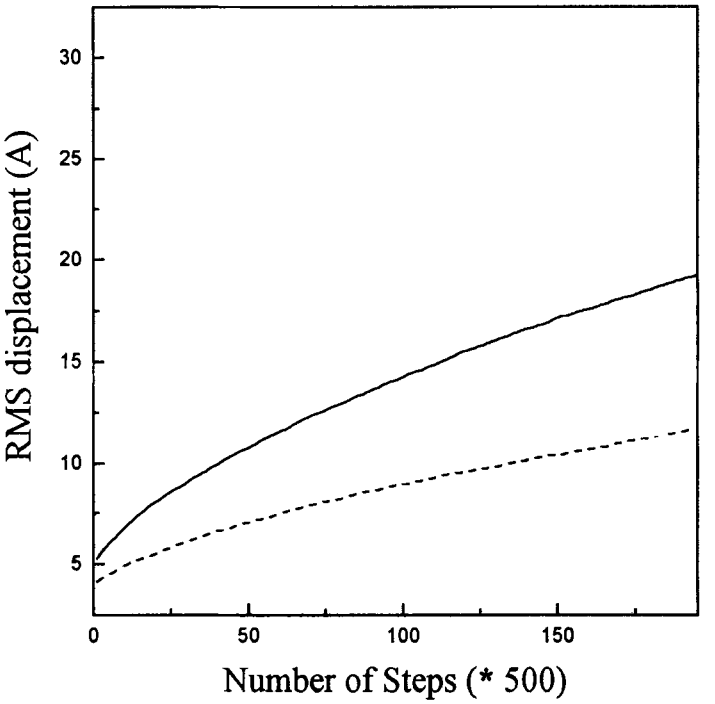

Figure 2 The root mean square displacement (RMS) of water molecules as a function of a number of MD steps. The solid line represents the *T*-denatured system and the dashed line the *P*-denatured one.

Table 2 The hydrogen bond of Ribonuclease A. These are obtained from the minimized solvated structures. We counted only hydrogen bonds with a length below 2.5 Å

	Ratio (%)	
	Intramolecular	*With Water*
Initial	36.93	63.07
P-denatured	25.42	74.58
T-denatured	29.97	70.03

of *T*-denatured. But, the protein-water hydrogen bond is increased more by *P*-denaturation than by *T*-denaturation.

In Table 3, the solvent accessible surface areas are reported for all the protein residues and six selected residues which are known to determine the protein compressibility with tryptophan [22], even if it is not widely accepted. The total area is increased more than the initial structure, and the degree is predominant in case of the T-denatured system. But, the value for the residues is decreased by the pressure while increased by temperature. The six compiled residues prefer the protein interior to its surface, and have a relatively lower contribution to the total surface area. The

Table 3 Solvent Accessible Surface Area of Ribonuclease A. The unit is in Å^2. The number in parenthesis is the number of corresponding residue existing in the protein

	Initial	*P-denatured*	*T-denatured*
Ala(12)	450.27	473.19	466.73
Val(9)	275.07	273.72	307.16
Leu(2)	92.59	95.96	89.30
Ile(3)	39.69	22.31	22.21
Phe(3)	52.02	34.91	59.71
Met(4)	76.53	92.32	156.55
6-residue(33)	1006.60	991.61	1101.03
total area(124)	6163.42	6304.37	6606.41

contribution for the *T*-denatured structure is larger than that for the *P*-denatured one. Compared with the residues of the initial structure, Leu and Phe residues show very different tendencies for the two cases, especially Phe. Figure 3 represents the solvent accessible surface area of these six residues by dots for the two systems. In Table 4, the surface area of the residues other than the above six residues are listed. The areas of Asp, Gly, Pro, and Ser are very different for the two systems.

Table 5 shows the occurrence of each ring and its cluster energy. The number of rings of the *P*-denatured system is much larger than that of the *T*-denatured. For the *T*-denatured system, the contribution of 3- and 4-rings to the total number of rings is relatively large compared with the *P*-denatured. As in our previous work [19], because our focus is on the 5-ring and 6-ring, we calculated the ratio of 5-ring to 6-ring. The

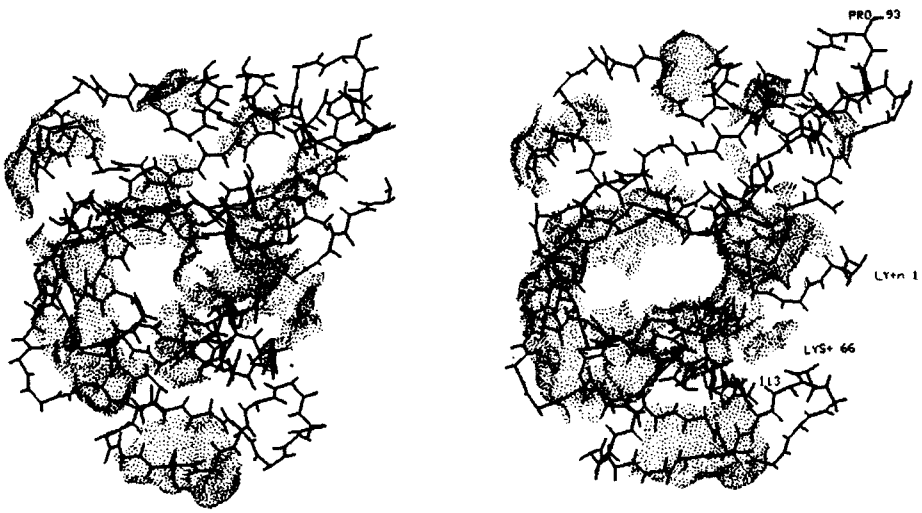

Figure 3 The solvent accessible surface area for the six residues, Ala, Val, Leu, Ile, Phe, and Met. The left side represents the *P*-denatured system and the right the *T*-denatured one.

Table 4 Solvent Accessible Surface Area of Ribonuclease A. The unit is in $Å^2$

	Initial	*P-denatured*	*T-denatured*
Arg(4)	335.87	397.75	388.75
Asn(10)	638.77	613.30	635.91
Asp(5)	223.13	203.00	289.91
Cys(8)	118.02	119.10	171.41
Gln(7)	417.36	451.82	432.70
Glu(5)	348.67	363.56	390.04
Gly(3)	172.82	154.96	177.33
His(4)	161.89	143.64	157.27
Lys(10)	789.69	830.51	875.21
Pro(4)	326.89	338.02	297.53
Ser(15)	806.71	768.52	820.56
Thr(10)	456.34	503.03	519.93
Tyr(6)	367.31	394.00	376.21

Table 5 The average occurence and cluster energy of each ring. The energy is in Kcal/mol. The value in parenthesis represents the ratio of each ring to the total number of rings

		P-denatured	*T-denatured*
Occurrence(%)	3-ring	$145.92 \pm 14.17(7.56)$	$103.20 \pm 10.82(9.56)$
	4-ring	$362.02 \pm 22.78(18.74)$	$233.36 \pm 16.57(21.62)$
	5-ring	$598.74 \pm 32.95(31.00)$	$332.65 \pm 21.02(30.82)$
	6-ring	$824.73 \pm 45.45(42.70)$	$410.14 \pm 27.16(38.00)$
Cluster energy	3-ring	-11.50 ± 0.32	-11.52 ± 0.16
	4-ring	-16.18 ± 0.19	-16.39 ± 0.22
	5-ring	-22.10 ± 0.19	-22.39 ± 0.23
	6-ring	-27.25 ± 0.20	-27.76 ± 0.27

ratios are 0.73 and 0.81 for the *P*- and *T*-denatured systems, respectively. This shows that the hydrophobic residues in protein surface are more exposed by thermal denaturation than by pressure denaturation, which is consistent with the solvent accessible surface area of the six selected residues.

In this article, we reported the thermodynamics and structure of the pressure and thermal denaturation for monomeric protein, Ribonuclease A in water, where we focused on the intramolecular interaction of protein and intermolecular interaction with water, not the protein-protein intermolecular interaction. Many proteins exist as dimers or higher under biological conditions and in more concentrated solutions where the protein-protein intermolecular interactions are critically affected by the pressure. The pressure effect on the dimer will be studied in the near future.

References

[1] G. Weber and H. G. Drickamer, "The effect of high pressure upon proteins and other biomolecules." *Q. Rev. Biophys.*, **16**, 89 (1983).

[2] A. E. Mark and W. F. Van Gunseteren, "Simulation of the thermal denaturation of hen egg white lysozyme: Trapping the molten globule state". *Biochemistry*, **31**, 7745 (1992).

[3] P. C. Michels, D. S. Clark, M. W. Adams and R. M. Kelly, *Biocatalysis at Extreme Temperatures: Enzyme Systems Near and Above 100°C*, American Chemical Society, Washington, DC, pp. 108–121 (1991).
[4] S. J. Gill and J. Glogovsky, "Influence of pressure on the reversible unfolding of ribonuclease and poly-γ-benzyl-L-glutamate". *J. Phys. Chem.*, **69**, 1515 (1965).
[5] S. Eshadri, K. A. Oberg and A. L. Fink, "Thermally denatured ribonuclease A retains secondary structure as shown by FT IR". *Biochemistry*, **33**, 1351 (1994).
[6] J. F. Brants, R. J. Oliveira and C. Westort, "Thermodynamics of protein denaturation. Effect of pressure on the denaturation of ribonuclease A". *Biochemistry*, **9**, 1038 (1970)
[7] R. F. Jr. Tilton, J. C. Dewan and G. A. Petsko, "Effects of temperature on protein structure and dynamics: X-ray crystallographic studies of the protein ribonuclease A at nine different temperatures from 98 to 320 K". *Biochemistry*, **31**, 2469 (1992)
[8] A. D. Robertson and R. L. Baldwin, "Hydrogen exchange in thermally denatured ribonuclease A". *Biochemistry*, **30**, 9907 (1991)
[9] T. R. Sosnick and J. Trewhella, "Denatured states of ribonuclease A have compact dimensions and residual secondary structure". *Biochemistry*, **31**, 8329 (1992).
[10] P. T. T. Wong and K. Heremans, "Pressure effects on protein secondary structure and hydrogen deuterium exchange in chymotrypsinogen: a Fourier transform infrared spectroscopic study". *Biochimica et Biophysica. Acta.*, **956**, 1 (1988).
[11] C. A. Royer, A. P. Hinck, S. N. Loh, K. E. Prehoda, X. Peng, J. Jonas and J. L. Markley, "Effects of amino acids substitutions on the pressure denaturation of staphylococcal nuclease as monitored by fluorescence and nuclear magnetic resonance spectroscopy". *Biochemistry*, **32**, 5222 (1993).
[12] D. B. Kitchen, L. H. Reed and R. M. Levy, "Molecular dynamics simulation of solvated protein at high pressure". *Biochemistry*, **31**, 10083 (1992).
[13] V. Daggett and M. Levitt, "A model of the molten state from molecular dynamics simulations". *Proc. Natl. Acad. Sci. USA*, **89**, 5142 (1992).
[14] A. Wlodawer, L. A. Svensson, L. Sjolin and G. Gilliland, "Structure of phosphate-free ribonuclease A refined 1.26 Å". *Biochemistry*, **27**, 2705 (1988).
[15] S. Toxvaerd, "Molecular dynamics at constant temperature and pressure". *Phys. Rev. E* **47**, 343 (1993).
[16] H. J. C. Berendsen, J. P. M. Postma, W. F. Van Gunsteren, A. Dinola and J. R. Haak, "Molecular dynamics with coupling to an external bath". *J. Chem, Phys.*, **81**, 3684 (1984).
[17] S. Lifson, A. T. Hagler and P. Dauber, "Consistent force field studies of intermolecular forces in hydrogen-bonded crystals: 2. A benchmark for the objective comparison of alternative force fields.". *J. Am. Chem. Soc.*, **101**, 5122 (1979).
[18] H. J. C. Berendsen, J. P. M. Postma, W. F. Van Gunsteren and J. Hermans *Intermolecular Forces* B. Pullman, eds, Reidel, Dordrecht, The Netherlands, pp. 331 (1981).
[19] J. Y. Yu, J. K. Shin and M. S. Jhon, "The structure of water in human ras oncogene proteins". *Int. J. Quan. Chem.*, **51**, 241 (1994).
[20] F. M. Richards, "Areas, volumes, packing, and protein structure". *Annu. Rev. Biophys. Bioeng.* **13**, 151 (1977).
[21] J. L. Silva and G. Weber, "Pressure stability of proteins". *Annu. Rev. Phys. Chem.*, **44**, 89 (1993).
[22] M. M. Gromiha and P. K. Ponnuswamy, "Relationship between amino acids properties and protein compressibility". *J. Theor. Biol.*, **165**, 87 (1993).