MOLECULAR DYNAMICS ANALYSIS OF BONE MORPHOGENETIC PROTEIN-2 CONFORMATIONS AND MECHANICAL PROPERTIES

IULIANA APRODU[a], IULIANA BANU[a], ADRIAN ISTRATE[a], EUGENIA VASILE[b], ANDREEA MADALINA PANDELE[b], EUGENIU VASILE[c], MARIANA IONITA[b*]
[a]*Dunarea de Jos University of Galati, Galati, 8002001, Romania*
[b]*University Politehnica of Bucharest, Bucharest, 060042, Romania*
[c]*Metav CD, Bucharest, 020011, Romania*

The knowledge of the dynamics and conformational particularities of bone morphogenetic protein-2 (BMP-2) are important for predicting its bioactivity in the process of osteoblasts differentiation and proliferation while using it in bone implants and scaffolds. Molecular dynamics simulations were performed to investigate the conformational stability of bone morphogenetic protein-2 in water environment at temperatures resembling the room and human body conditions. According to our results, BMP-2 behaviour is slightly influenced by small temperature fluctuations. The temperature increase caused the reduction of the strands and the increase of helices content. In addition steered molecular dynamics simulations were performed to stretch the protein up to 10% elongation and the results indicate that BMP-2 exhibits a more rigid behaviour at room temperature.

(Recieved November 15, 2012; Accepted December 20, 2012))

Keywords: Bone morphogenetic protein 2, Molecular dynamic, Conformational change, Mechanical properties

1. Introduction

Because of the large number of degenerative disc disease, osteoarthritis, and osteoporosis, most of the latest researches on orthopaedic field were focused on different alternatives for bone grafting [1].

Bone tissues have good regenerative abilities in normal physiological conditions of the human body. The success of tissue engineering highly depends on the progress in the field of biotechnology of morphogenetic factors and biomaterials. Tissue engineering experiments consisting on the replacement and regeneration of lost or destroyed tissues, require precursor cells from the patient with scaffolding matrices and the stimulus of growth factors [2]. One of the most important growth factors in bone formation and healing are bone morphogenetic proteins (BMPs) [2-3]. The three-dimensional structures of BMPs from various sources have been determined mainly by X-Ray diffraction and different forms were found to share certain similarities. Among BMPs, most of the solved structures are related to bone morphogenetic protein-2 (BMP2) which is a highly conserved homodimeric protein of about 70 Å x 35 Å x 30 Å size [4]. In animal cells the BMP2 molecules is glycosilated, probably at ASN^{56} residue [5]. It has been shown that BMP2 is osteoinductive and to potently induce osteopath differentiation in different types of cells [6]. Each monomer have a thick part in the middle corresponding to one β-sheet layer, and contains a cystine-knot consisting on six cysteine residues that interact to form three disulfide bridges (Cys^{14}-Cys^{79}, Cys^{43}-Cys^{11} and Cys^{47}-Cys^{113}). The cystine-knot plays important role in BMP2 structure stabilization, as the protein lacks the common hydrophobic core [4, 7].

*Corresponding author: mariana_ionita2003@yahoo.com

In order to better understand the morphogenetic signal and host cells response in bone regeneration, the knowledge of BMPs at single protein level is very important. The present work consists of a computational study focused on conformational particularities of BMP-2 and its mechanical response to external forces. The protein behaviour at different temperatures was estimated at single molecule level, using the *in silico* approach. In the last years the molecular dynamics (MD) simulations have been widely used to study biomolecules behaviour [8] therefore allowing deeper understanding of different phenomena related to molecular motion or interactions.

2. Materials and methods

2.1 Molecular model

The crystal structure of human bone morphogenetic protein-2 was obtained from the Brookhaven Protein Data Bank. The atomic coordinates of 3BMP.pdb at 2.7 Å resolution [4] were used to investigate the conformational particularities of BMP-2.

The gene sequence P12643 (UniProtKB/Swiss-Prot) obtained from *Homo sapiens* have provided the primary structure for the protein. The chosen structure was refined before use by removing the (4S)-2-methyl-2,4-pentanediol which is not present under physiological conditions and all water molecules.

2.2 Numerical set-up

Gromacs 4.5.5 software [9] with the GROMOS96 43a1 force field was used to perform all energy minimizations and molecular dynamics (MD) simulations in parallelization conditions on a Intel(R) Core(TM)2 CPU 6300 1.86 GHz processor-based machine running Linux.

The protein was first energy minimized in vacuum to ensure that the investigated molecule was free of strongly repulsive non-bonded contacts or geometric distortions inconsistent with the potential energy function [10].

The minimized structure was centred in a rectangular box of 79.12 Å x 79.12 Å x 79.12 Å with periodic boundary conditions, and the rest of the box was filled with single point charge (SPC) explicit water molecules to model the protein-water interactions. The final system consisting of 49033 atoms (including 15992 water molecules) was afterwards prepared for the MD steps by performing an additional energy minimization.

Both energy minimization steps were carried out using the following two algorithms in sequence: steepest descent and limited-memory Broyden-Fletcher-Goldfarb-Shanno.

To understand the atomic details of the BMP-2 behaviour, different MD steps were performed to gradually increase the temperature to 25 and 37°C by coupling each component of the system (protein and water molecules) to a Berendsen thermostat for 100 ps. A 1 ns equilibration step was then performed to remove any potential temperature and energy oscillations of the system heated at the above mentioned temperatures.

The time step used in all MD simulations was 0.001 ps; the electrostatic interactions were treated with the Particle-Mesh-Ewald (PME) method with a Coulomb cut off of 1.7 nm, Fourier spacing of 0.12 nm and a fourth order interpolation; the van der Waals' interactions were treated using Lennard-Jones potential and a switching function with a cutoff distance of 1.1 nm and a switching distance of 0.9 nm.

The structural particularities of the equilibrated systems were investigated by means of PDB sum tool.

2.3 Mechanical characterization of BMP-2

The models equilibrated at 25 °C and 37 °C were further used for the evaluation of the mechanical properties of BMP-2. The set up used was inspired by the *in vitro* experimental techniques. The protein was stretched up to 10% elongation by performing Steered Molecular

Dynamics (SMD) simulations to investigate the molecular response to Atomic Force Microscopy tests.

The protein was elongated by displacing the pull group (P) consisting of the C_α atoms of the aminoacid Asn^{95} while freezing the reference group (R) consisting of the C_α atoms of the aminoacid Val^{70} (Fig. 1). The P group was pull at a constant rate of 0.001 nm ps^{-1} by means of a harmonic spring attached to its centre of mass. In order to limit the computing costs and to insure the accuracy of the results obtained a spring elastic constant (*Ks*) of 3000 kJ mol^{-1} nm^{-2} was used [11].

The stiffness of the BMP-2 was estimated as the slope of the linear fitting of the strength-elongation curve.

3. Results and discussion

3.1 Analysis of the equilibrated structures

In order to gather information at the atomic level on molecular behaviour of BMP-2 the *in silico* approach was used.

The BMP-2 model was minimized, solvated and equilibrated at temperatures resembling the room temperature (25°C) and human body conditions (37°C). The potential energy of the refined BMP-2 structure was $-8.98 \cdot 10^5 \pm 39.03$ kJ/mol after energy minimization in aqueous environment, lower compared to the potential energy of the protein after the initial energy minimization in vacuum ($-5.33 \cdot 10^3 \pm 16.53$ kJ/mol).

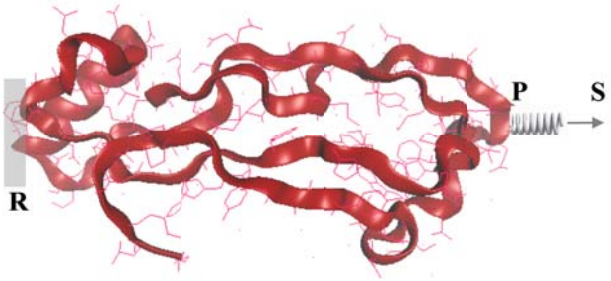

Fig. 1. Atomic structure of BMP-2. Scheme of the tests mimicking the optical tweezers set-up. The protein was stretched by freezing the R group consisting on C_α atoms of Val^{70}, while pulling the P group consisting on C_α atoms of Asn^{95} at a constant rate of 0.001 nm ps^{-1} by means of a spring S.

After the temperature increase and about 600 ps of equilibration dynamics, the root mean square deviation (RMSD) of the Cα atom position with respect to the starting structure reached the stable value of 0.29±0.01 nm (mean±SD), and 0.39±0.05 nm, respectively in the case of system equilibrated at 25°C and 37°C.

The reliability of the model equilibrated at room and human body temperature was established based on the stereochemical quality of the molecules checked by means of PROCHECK. The detailed overall and residue-by-residue geometry analysis based on bond lengths, angles, and dihedral angles was performed for initial and equilibrated structures, and the results indicated an improvement of each parameter in case of the equilibrated structures of BMP-2. The Ramachandran plots (Fig. 2) were used to evaluate the quality of the starting structures and consequently the reliability of the obtained results [12]. The high percentage of residues falling in the permitted regions of the Ramachandran plot (Table 1) indicates the good reliability of the obtained results.

3.2 Conformational analysis

The equilibrated models were further characterized in terms of conformational [13] and mechanical properties.

Table 1. Conformational details of the BMP-2 model equilibrated at 25 °C and 37 °C

	25 °C	37 °C
Fully allowed regions (%)	77.2	69.6
Additionally allowed regions (%)	17.4	25.0
Generously allowed regions (%)	4.3	4.3
Disallowed regions (%)	1.1	1.1

It is generally accepted that protein behaviour is highly influenced by the temperature. The root mean square error obtained by least squares fitting the BMP-2 models equilibrated at 25 °C and 37 °C was 5.34 Å is indicating important conformational rearrangements at human body temperature with respect to the room temperature.

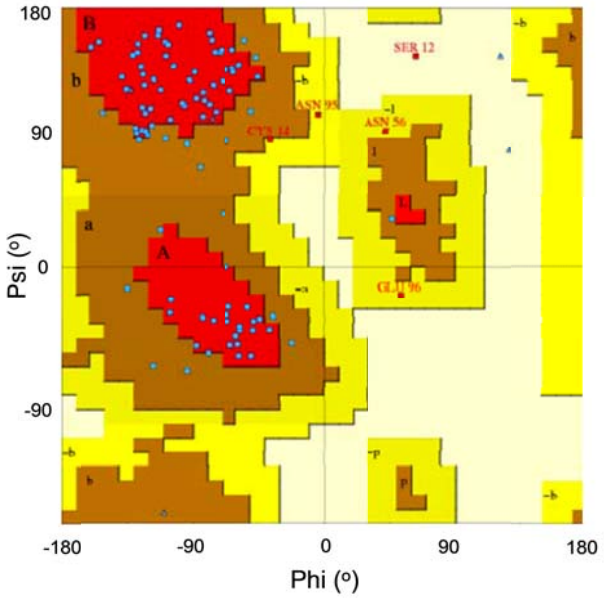

Fig. 2. Ramachandran plot of the BMP-2 model equilibrate at 25°C. Red, brown and yellow mark the fully, additionally and generously allowed regions, respectively.

Analyzing the results presented in Table 2 one can see that the conformational changes induced by the temperature are related both to the strands and helices content. The temperature increase from 25 °C to 37 °C causes the reduction of the strands and the increase of helices content within protein structure. Regardless of temperature, the secondary structure is dominated by the strand motif. The strands within BMP-2 structure are organized within three parallel beta sheets representing 40.6% of the protein secondary structure at 25 °C and 26.4% at 37 °C (Table 2). Our results comply with the observation of Scheufler *et al*. [4] who provided molecular details on BMP-2 molecule after crystallization. One of the β-sheets has a twisted crossover conformation and is closed to a α-helix which is almost perpendicular to its strands. Moreover, Daoppin *et al*. [14] describes the overall BMP2 structure as a hand where the cystine-knot core forms palm, while the helix and β-sheets are the wrist and fingers, respectively. At both tested temperatures the loop connecting the strands β7 and β8 has a hairpin like conformation as reported by Sibanda and Thornton [15] for both BMP2 and BMP7.

The residue Phe^{41} is considered very important for the stabilization of the protein secondary structure involving the β2, β5, β6 and β9 strands which are arranged close to form a four-stranded antiparallel β-sheet [4]. In case of both studied temperatures, the PROCHECK

analysis [16] indicate that this residue have low values for the absolute deviation from mean of Chi-1 value and omega torsion. No hydrogen bonds involving Phe^{41} could be identified in the structures equilibrated at 25 and 37°C.

Although the total number of helices formation is the same at tested temperatures, only the ~15 Å long helix (Asn^{59}-Asn^{68}) could be identified in both structures due to the low stability the helices. Moreover, at human body temperature the right-handed 3-10 helix structure disappears.

The content of unordered secondary structure varied significantly with the temperature; a 13.2% decrease of the elements of regular secondary structure was obtained at human body temperature with respect to the room temperature.

The unordered secondary structure was dominated by the beta turns motifs: the total number of beta turns decreased from 13 at 25 °C to 12 at 37 °C, whereas the number of gamma turns increased from 1 to 7.

Table 2. Secondary structure details of BMP-2 model equilibrated at 25 °C and 37 °C

	25 °C	37 °C
Beta sheets	3 antiparallel beta sheets: A with 2 strands (Lys^{15}-Val^{21}; Tyr^{38}-His^{44}), B with 3 strands (Ile^{32}-Ala^{34}; Leu^{84}-Tyr^{91}; Val^{99}-Val^{108}) and C with 2 strands (Cys^{78}-Pro^{81}; Cys^{111}-Arg^{114})	3 antiparallel beta sheets: A with 2 strands (Lys^{15}-His^{17}; Tyr^{42}-His^{44}), B with 2 strands (Val^{80}-Leu^{84}; Val^{108}-Gly^{112}) and C with 2 strands (Ile^{87}-Leu^{92}; Val^{98}-Tyr^{103})
Strand (%)	40.6	26.4
No. of helices	2 (Asp^{53}-Leu^{55}; Asn^{59}-Asn^{68})	2 (Phe^{23}-Val^{26}; Asn^{59}-Asn^{68})
α-helix (%)	9.4	13.2
3-10 helix (%)	2.8	0.0

Table 3. Structural indicators of BMP-2 model equilibrated at 25 °C and 37 °C

Temperature	Hb within the protein	Biffurcated Hb within the protein	Hb water - protein	HSAS, nm^2	Protein-water interaction energy, kJ/mol
25 °C	67	10	223	43.26	-15754.2
37 °C	64	6	217	42.12	-16114.5

The stability of the protein is given by the compensating forces acting between residues, such as favourable hydrophobic, van der Waals and hydrogen bonding and unfavourable conformational entropy [17].

The investigation of the protein hydrogen bonding and hydrophobic surface available to the solvent was carried out on the molecular dynamics trajectory data.

All changes in BMP2 structure, as well as the interactions established with the water environment highly influence the molecular behaviour of the protein [18]. The temperature increase from 25 to 37°C causes a slight decrease of the hydrophobic surface available to the solvent (HSAS) which was calculated as the van der Waals' envelope of the proteins expanded by the radius of the solvent sphere about each solute atom centre. The HSAS represents 58-59% of the total solvent accessible surface of the protein.

Hydrogen bonds (Hb) were computed based on geometrical features between acceptor (A) and donor (D) atoms using HBPLOT program [19-20]. The hydrogen bonds were identified based on the following criteria: D-A distance < 3.9 Å, H-A distance < 2.5 Å, D-H-A angle > 90^0, D-A-AA angle > 90^0 and H-A-AA angle > 90^0, where AA is the atom attached to the acceptor.

The pattern detailed hydrogen-bonding interactions is a function of time. The number of hydrogen bonds within the protein and between the protein and water molecules was evaluated. The small conformational changes occurring in the BMP2 with the temperature caused variation

on the simple and bifurcated Hb number (Table 3). McDonald and Thornton [19] showed that bifurcated hydrogen bonds are weaker than single bonds since the interaction energy associated does not double but increases by only 58%.

Most of the hydrogen bonds have hydrogen - acceptor distances ranging from 2.7 to 3.0 Å and connect the main chains of two different residues (~73%); only 7% of Hbs are established between the side chains of aminoacids, and the rest are side-main chain type. Regardless of the simulation conditions, the Hbs are involved in the stabilization of each secondary elements of the protein structure [21].

A detailed check of the Hb involved in the stabilization of the BMP2 structure showed that ~ 23% of the bonds ensure β-sheet regions packing, while ~34% are responsible for α-helix backbone effective stabilization at 37 ^{o}C.

The Hbs involved in the stabilization of β-sheets are responsible for shielding effect, leading to relatively strong interactions that might be responsible for slow dissolution of β-sheet structure once formed, as well as for protein misfolding [22].

3.3 Analysis of mechanical properties

The molecular response of the BMP2 to typical optical tweezers tests, simulated by stretching the protein up to 10 % elongation, was analysed by plotting the force versus the protein elongation. Figure 3 presents the results of SMD simulations performed at constant pulling rate of 0.001 nm/ps and with a spring constant of 3000 kJ/(mol nm^2). These parameters have been chosen based on previous sensitivity analysis [11] showing that higher spring stiffness reduces the system oscillations and are hence more effective in terms of computing costs and lower pulling rates are associated to higher oscillating motion of the system and higher protein stiffness.

The value of the pulling rate is usually established based on the size of the molecular systems. The pulling rate used in the present experiment was comparable to the one adopted in previous computational studies but higher compared to the pulling rates used regularly in AFM experiments [23] that varies in the nm/ms range.

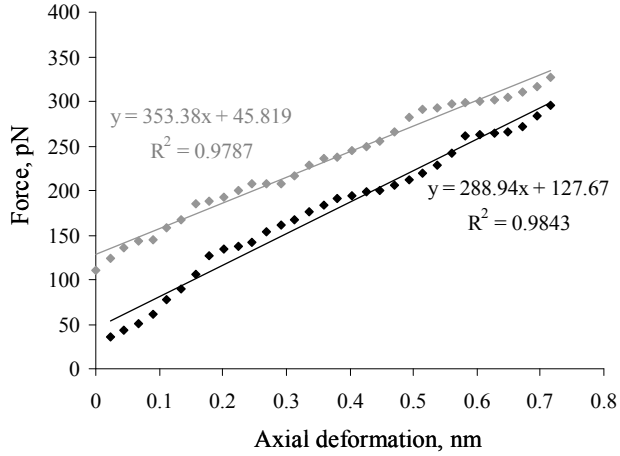

Fig. 3. Force vs. molecular elongation for BMP2 elongation tests performed at a pulling rate of 0.001 nm/ps with a spring constant k_S = 3000 kJ/(mol nm^2).

The stiffness of BMP2 was estimated as the slope of linear fitting of the force experimented by the P group due to the action of the spring *vs.* protein elongation. As shown in Figure 3, BMP2 conformation seems to be more flexible at human body temperature (stiffness of 288.94 pN/nm), compared to the room temperature (stiffness of 353.38 pN/nm). The value of the stiffness obtained in our study, by performing SMD simulation for the BMP2 is comparable with the elasticity reported in the literature for two globular protein, myosin and kinesin [11].

4. Conclusion

In this study, the *in silico* approach was used to simulate the dynamics of BMP2 in water-like medium and to estimate its conformational particularities at room and human body temperature. Computational results show that small conformational changes, in the order of few Angstrom, occurring in the BMP-2 structure reflect on its mechanical properties. The knowledge of the conformational particularities of the BMP2 molecules in different conditions might allow a better understanding of its structure-function relationship. In order to get a more complete picture on role of BMP-2 in bone healing, further investigations are to be performed for identifying the specificity of the protein for different other tissue components.

Acknowledgements

This research has been supported by the National Research Grant PN-II-PCCA-140, CNCS-UEFISCDI.

References

[1] M. Geiger, R. H. Li, W. Friess, Adv. Drug Deliv. Rev. **55**, 1613 (2003).
[2] P.C. Bessa, M. Casal, R.L. Reis, J. Tissue Eng. Regen. Med. **2**, 81 (2008).
[3] A. H. Reddi, Growth Factor Rev. **16**, 249 (2005).
[4] C. Scheufler, W. Sebald, M. Hulsmeyer. J.Mol.Biol. **287**, 103 (1999).
[5] D. I. Israel, J. Nove, K. M. Kerns, I. K. Moutsatsos, R. J. Kaufman, Growth Factors. **168**, 139 (1992).
[6] P. J. Marie, F. Debiais, E. Haÿ, Histol. Histopathol. **17**, 877 (2003).
[7] T. Kirsch, J. Nickel, W. Sebald. EMBO J. **19**, 3314 (2000).
[8] A. Gautieri, M. Ionita, D. Silvestri, E. Votta, S. Vesentini, G. Fiore, N. Barbani, G. Ciardelli, A. Redaelli, J. Comput. Theor. Nanosci. **7**, 1287 (2010).
[9] E. Lindahl, B. Hess, D. van der Spoel, J. Mol. Mod. **7**, 306 (2001).
[10] I. Aprodu, N. Stănciuc, I. Banu, G. Bahrim, J. Sci. Food Agric. DOI: 10.1002/jsfa.5799.
[11] I. Aprodu, M. Soncini, A. Redaelli, Macromol. Theory Simul. **17**, 376 (2008).
[12] G. N. Ramachandran, V. Sasisekharan. Adv. Protein Chem. **23**, 283 (1968).
[13] R. A. Laskowski, Nucleic Acids Res. **37**, 335 (2009).
[14] S. Daopin, K. A. Piez, Y. Ogawa, D. R. Davies. Science. **257**, 369 (1992).
[15] B. L. Sibanda, J. M. Thornton, Methods Enzymol. **202**, 59 (1991).
[16] A. L. Morris, M. W. MacArthur, E. G. Hutchinson, J. M. Thornton, Proteins. **12**, 345 (1992).
[17] B. M. P. Huyghues-Despointes, N. C. Pace, W. S. Englander, J. M. Scholtz, Methods Mol. Biol. **168**, 69 (2001).
[18] F. Eisenhaber, P. Argos, Prot. Eng. **9**, 1121 (1996)
[19] I. K. McDonald, J. M. Thornton, J. Molec. Biol. **238**, 777 (1994).
[20] Z. Bikadi, L. Demko, E. Hazai, Arch. Biochem. Bioph. **461**, 225 (2007).
[21] G. Rose, P. Fleming, J. Banavar, A. Maritan, PNAS. **103**, 16623 (2006).
[22] J. E. Shea, C. L. Brooks III, Ann. Rev. Phys. Chem. **52**, 499 (2001).
[23] P. F. Lenne, A. J. Raae, S. M. Altmann, M. Saraste, J. K. H. Horber, FEBS Letters. **476**, 124 (2000).