Dedicated to Professor Mircea Diudea
on the Occasion of His 65th Anniversary

NATURE OF POLYETHYLENEIMINE-GLUCOSE OXIDASE INTERACTIONS

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ABSTRACT. The nature of interactions between Polyethylenimine and glucose oxidase 3QVR enzyme was studied by docking and Molecular dynamics procedures. The docking procedure evidenced two active sites, one located inside the enzyme body and the second at the enzyme surface. In view of deeply understand the interactions ligand – enzyme, a Molecular dynamics study followed to the docking. This provided a detailed information on the type, intensity and frequency of these interactions.

Keywords: PEI, 3QVR, Glucose oxidase (GOx), Docking, Molecular Dynamics

INTRODUCTION

Glucose oxidase (GOx) is an enzyme produced by several fungi, such as *Aspergillus*, *Penicillium* and other species. It catalyzes the oxidation of beta-D-glucose to D-glucono-1,5-lactone, which further hydrolyzes to produce the gluconic acid. A coproduct of this enzymatic reaction is the hydrogen peroxide (H_2O_2) [1-3]. GOx has found several applications in Chemistry and Pharmaceutics, in production of biosensors that use this enzyme immobilized on different nanomaterials, like the carbon nanotubes and/or biopolymers such as polyethylenimine PEI, polyethileneglicol PEG, polyaminoamide PAMAM, etc [4-6].

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The problem of GOx immobilization is to retain the enzyme native activity despite its immobilization onto the polymer surface. In this respect, knowledge of the intimate interaction between the enzyme and ligands, in vary conditions, is of great importance.

In this paper, a coupled study of docking and Molecular Dynamics was conducted and the results were collected and interpreted.

METHODS

Docking Procedure

Using the AutoDockVina software [7], the protein molecule was loaded and stored as "protein.pdb" after assigning hydrogen bonds [8]. The investigated ligand was loaded and its torsions along the rotatable bonds were assigned; then the files were saved as "ligand.pdbqt". The grid menu is next toggled [9]; after loading "protein.pdbqt", the map files were selected directly with setting up the grid points, for the search of ligand-protein interactions. The enzyme allows several interactions with ligands, that's why a map of ligand - enzyme interactions was made, for all area of enzyme, by changing parameters of grid box, step by step. The docking parameter files were completed by using the Lamarckian genetic algorithm [10].

Molecular Dynamics Method

A Molecular Dynamics (MD) procedure was applied to a complex enzyme–ligand [11]. The structure of ligand and enzyme was characterized by using the Amber force field parameters; the atomic charges were calculated according to the Merz-Kollmann scheme, via the RESP procedure [12] at HF/6-31G* level of theory. Each system was neutralized and immersed in a periodic TIP3P water box. The considered systems were heated up to 300K by 100 ps of initial MD simulation, while the temperature was controlled by the Langevin thermostat [13]. The periodic boundary conditions and SHAKE algorithm [14] were applied to 70 ns of molecular dynamic simulation: the first 10 ns of the simulation time was considered as the equilibration interval while the next 60 ns of trajectory were used for the analysis of interactions ligand-enzyme. Structural analysis was performed by the VMD package [15]. The energetic characterization of the interactions between the ligand and active sites was obtained by the Molecular Mechanic/Poisson-Boltzmann Surface Area (MMPBSA) method [16]. In all MD simulations, the AMBER 11 package [17] was used.

RESULTS AND DISCUSSION

Docking results

Based on crystal data, one can see that 3QVR/GOx enzyme can establish many interactions with a ligand, in all its area (Figure 1) [11]. That's why a docking study of PEI - 3QVR complex was made in all area of GOx enzyme, by changing parameters of grid box, step by step.
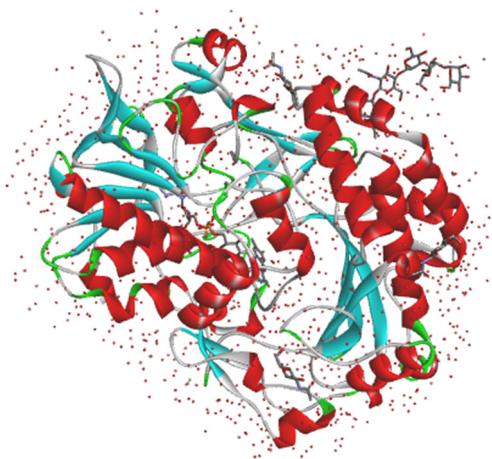

Figure 1. The protein 3QVR
(RCSB PDB, GOx - molecule of the month, May, 2006) [11]

As ligands, three types of PEI molecules were considered: branched (B), linear (L) and dendrimer (D) (Figure 2).

L-PEI   B-PEI   D-PEI
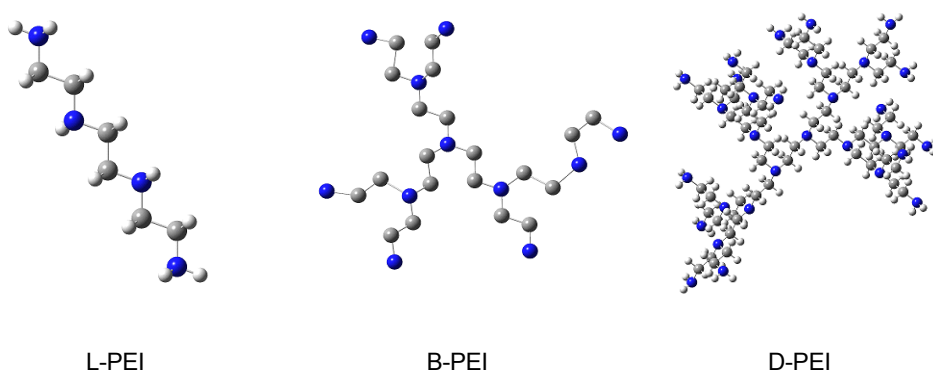
Figure 2. Examples of PEI molecules: linear L (left);
branched B (middle) and dendrimer D (right).

Among these 3 groups, the strongest affinity to GOx enzyme shows B-PEI (mean value -7.9 kcal/mol - Figure 3). The groups of ligands D-PEI and L-PEI exhibit less affinity to enzyme, in comparison to B-PEI (Figure 3). Only D3-PEI-C26N14 and L-PEI-C26N14 exhibit an affinity close to the ligands of B-PEI group (at about -7 kcal/mol) (Figure 3); the rest of ligands of the two groups D-PEI and L-PEI exhibit similar values, at about -5kcal/mol.
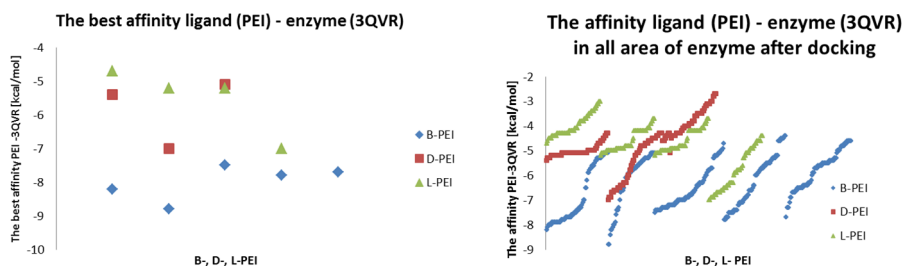
Figure 3. Energy (in kcal/mol) of the best affinity ligand (PEI) - enzyme (3QVR) complex, (left), and energy of all the docked ligand (PEI) - enzyme (3QVR) complexes, in all area of the enzyme (right), for B-, D-, and L-PEI.

Therefore, to simulate the DM, only one ligand of the B-PEI structures, namely the polymer PEI_C14N8_07_B22 (Figure 4) was used.
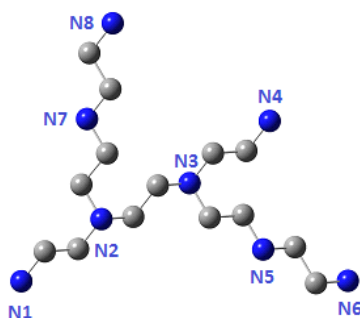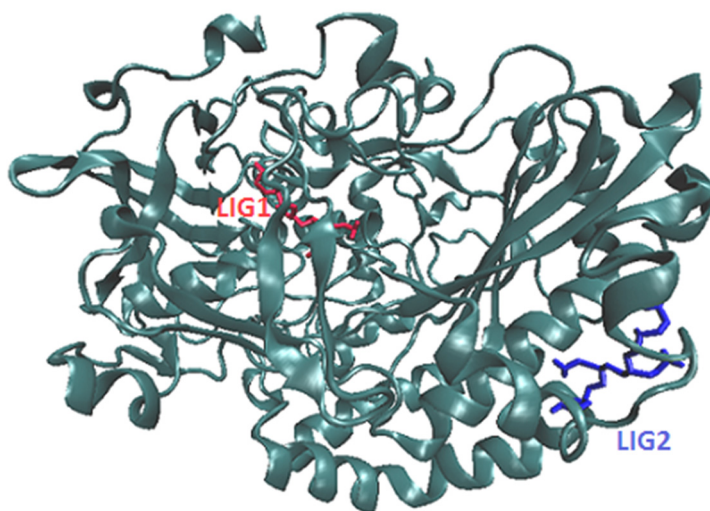

Figure 4. The ligand PEI_C14N8_07_B22.

The values of binding free energy were collected in the vicinity of the protein active site, where the ligand PEI_C14N8_07_B22 (Figure 4) was docked; data are listed in Table 1.

Table 1. The best binding energy of ligand PEI_C14N8_07_B22 at the active sites LIG1 and LIG2 of 3QVR, during the nine explored conformations.

Ligand binding site	1	2	3	4	5	6	7	8	9	Best docking Energy (kcal/mol)
LIG1	-5.8	-5.8	-5.6	-5.6	-5.6	-5.5	-5.5	-5.5	-5.5	-5.8
LIG2	-4.5	-4.3	-4.2	-4.2	-4.2	-4.2	-4.2	-4.1	-4.1	-4.5

Based on the best docked energies: -5.8 and -4.5 kcal/mol, two sites were differentiated on the protein body, which show the best affinity to PEI (Table 1). In the first case, the ligand is bound to amino acids inside of the protein area (LIG1, Figure 5), while in the second case the binding takes place outside of the enzyme surface (LIG2, Figure 5). That's why the Molecular Dynamics will be performed for the first and second active places of this protein, separately. Thus, the docking study gave information about the type and number of interactions between ligand and amino acid residuals of the enzyme. The results obtained during the docking procedure became the starting point in molecular dynamics study.

Figure 5. Two sites of the most important interaction ligand - enzyme (3QVR): inside of the protein, named LIG1, and on its surface, named LIG2. As a ligand, the molecule PEI_C14N8_07_B22 was used (see Figure 4).

Molecular Dynamics results

The Molecular Dynamics simulations allow to study the interactions within the enzyme – ligand complex, in the natural environment and in a large number of conformations of this complex, as well [18]. During MD, the time evolution of ligand PEI_C14N8_07_B22 lying in two active sites of the conformational space of 3QVR protein, inside (LIG1) and on its surface (LIG2) (Figure 5) was followed. The 60 ns of collected trajectories were used for structural analysis. In both cases, the first 10 ns of MD simulation show considerable fluctuations, while in the remaining time of simulation, the trajectories seem stabilized, meaning the equilibrium stage was attained (Figure 6).
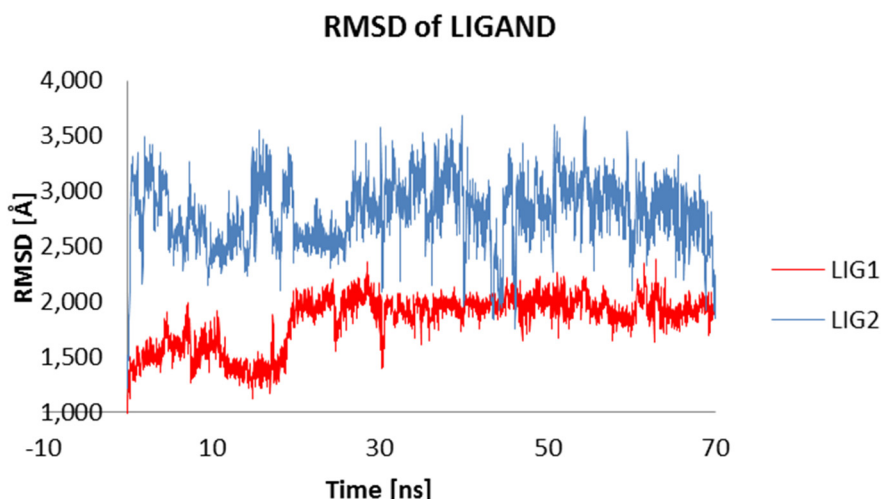

Figure 6. RMSD distribution of ligand: values characterizing the ligand interaction at the first active site (LIG1, red color) of 3QVR enzyme, inside the protein and at the second active site (LIG2, blue color), on the protein surface.

In case of complex located inside the protein body (LIG1, Figure 5) the MD simulation found five amino acids: ALA287, LEU27, SER101, SER94 and SER289 that interact with the ligand and stabilize the complex 3QVR – PEI; in case of complex located outside, on the enzyme surface (LIG2, Figure 5) only three amino acids ASN471, ASP358 and GLU354 have been found. In the first case, the number of formed hydrogen bonds HB is six while in the second, this number is seven.
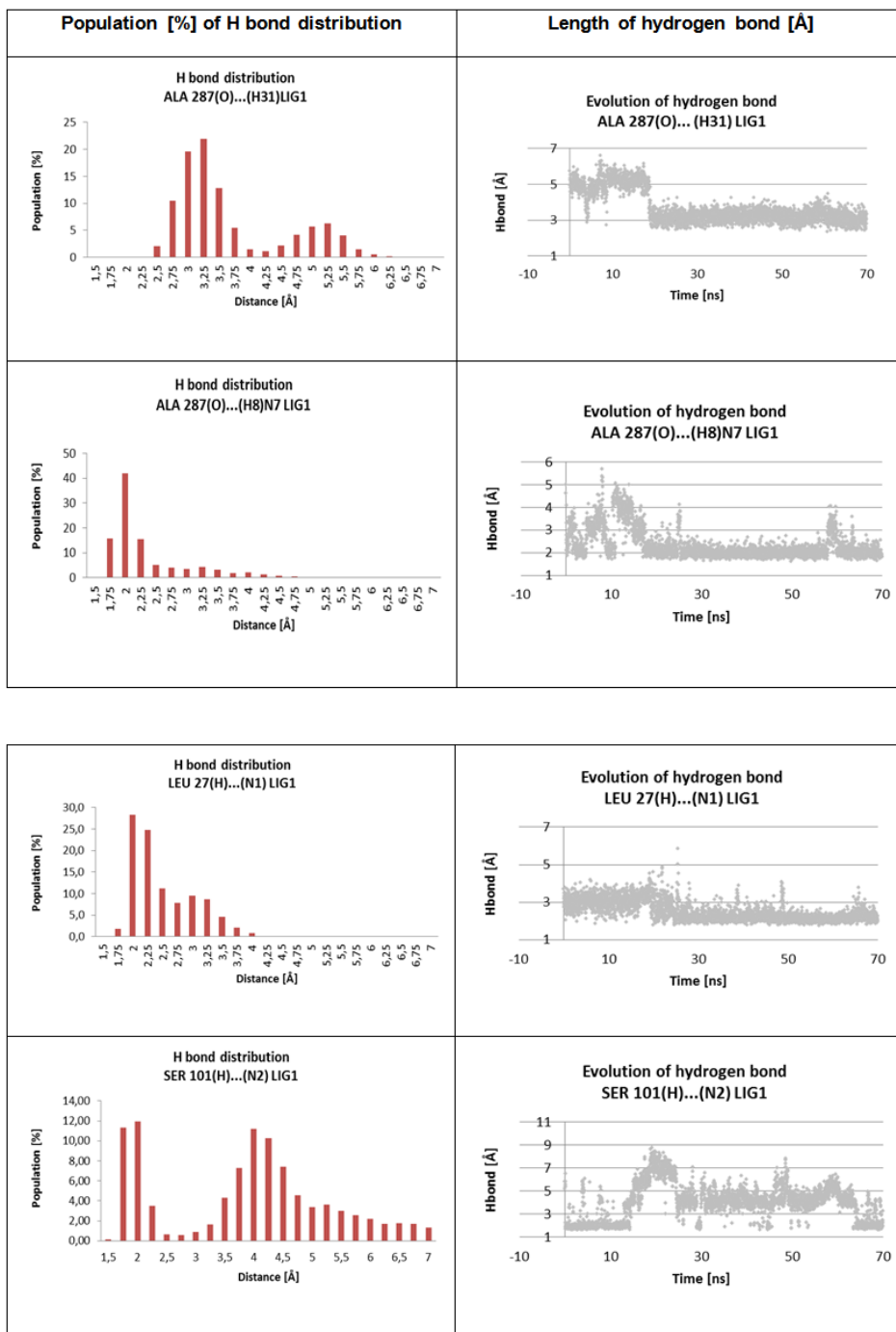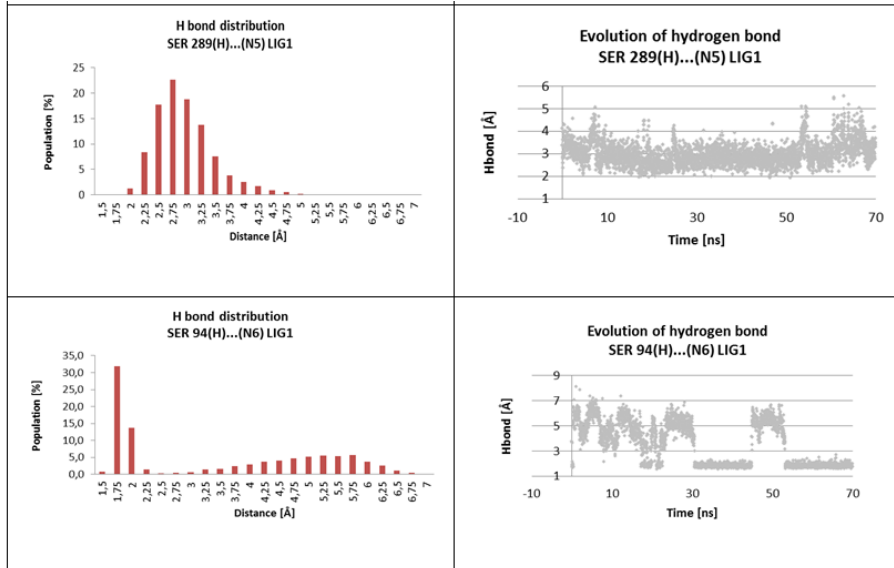
Figure 7. Distribution of hydrogen bonds length during the MD simulation time, created by PEI_C14N8_07_B22 with selected amino acids from the LIG1 active site of 3QVR inside of this protein (left) and evolution of hydrogen bonds as a function of time [ns] of MD.
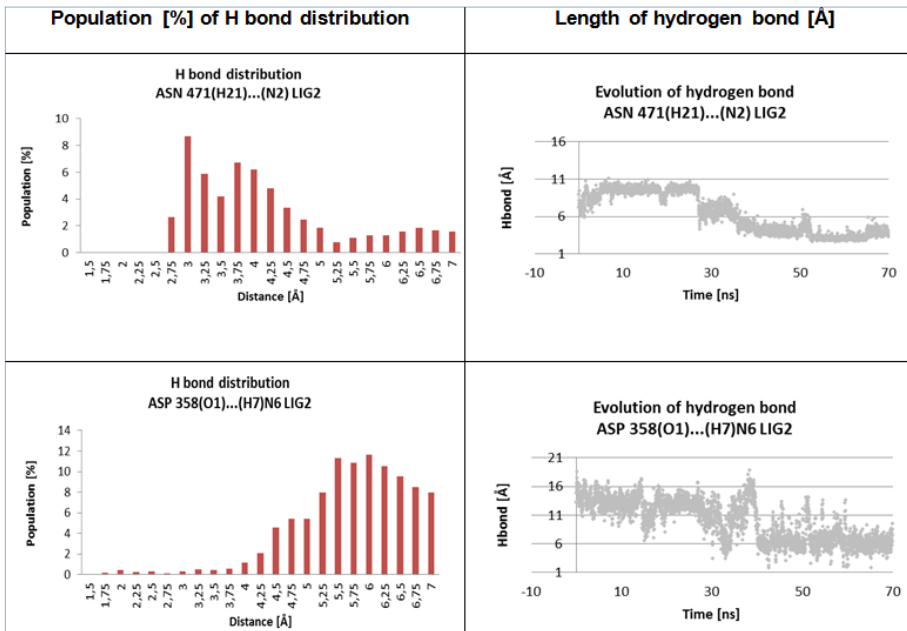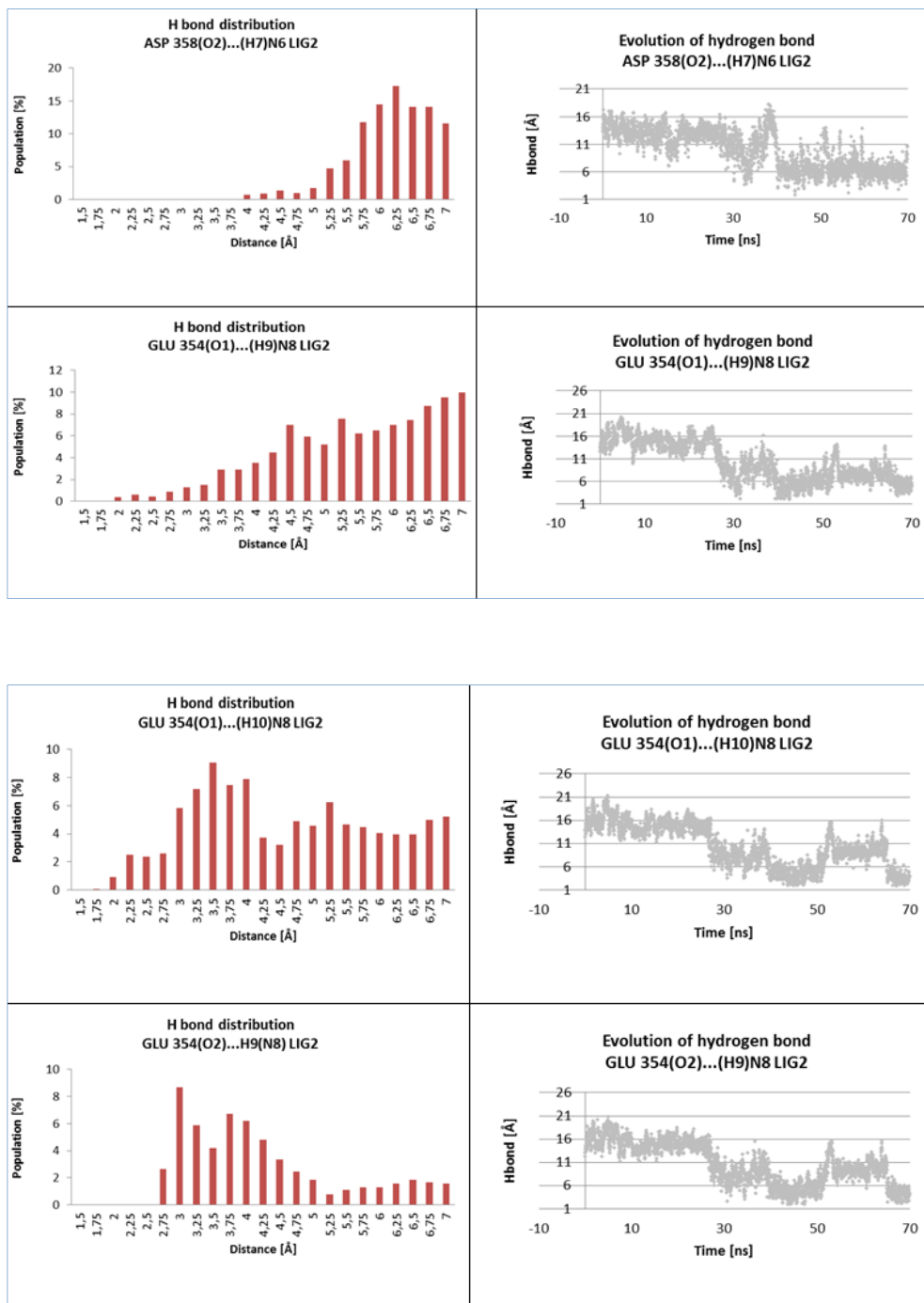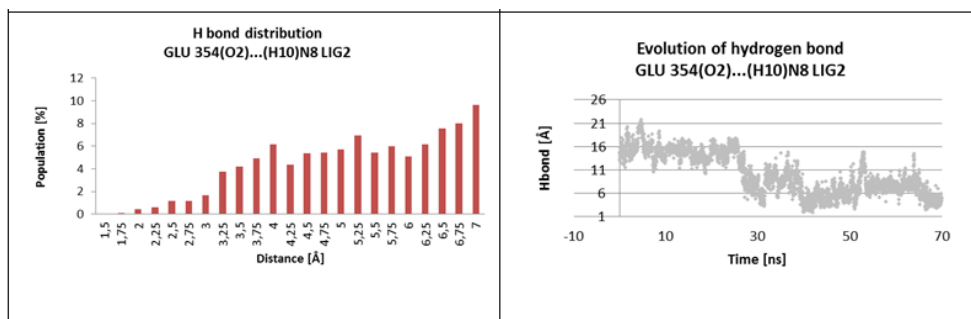
Figure 8. Distribution of hydrogen bonds length during the MD simulation time, created by PEI_C14N8_07_B22 with selected amino acids from the LIG2 active site of 3QVR on the surface of this protein (left) and evolution of hydrogen bonds as a function of time [ns] of MD (right).

In the first active side, LIG1, (Figure 7) of enzyme, there are five different aminoacids: ALA 287, LEU 27, SER 101, SER 289, SER 94 that form HBs in their interaction with the ligand (PEI_C14N8_07_B22). In case of ALA 287, LEU 27 and SER 101, such interactions appear at about 20 ns of the trajectory while in case of SER 289 they occur during all the time of simulation (Figures 7). Only the behavior of SER 94 is quite different ((Figure 7, bottom). Here HB appear from 30 ns of MD simulation but in the meantime disappear for about 10 ns. One can see the formation of strong HB (1.7Å) from 30 to 40 ns and next from 50 to 70 of simulation of DM. Aminoacid ALA 287 creates two HBs: ALA(O)....(H31) LIG1 and ALA(O)....(H8) LIG1 with ligand PEI_C14N8_07_B22, of the length 2.75-3Å (with 3Å having the largest distribution) and 1.75 Å -2.25 Å (with 2Å the largest distribution), respectively (Figures 7, left).

For other aminoacids: LEU27, SER101, SER289 and SER 94, the interactions of ligand PEI_C14N8_07_B22 with different N atoms and different H atoms of the amino acids in the enzyme are strong and medium ones (Figures 7, left). H atom of LEU27 creates a hydrogen bond with the nitrogen atom N1 of length 2Å-2.25Å (LEU 27(H)...(N1) LIG1). In case of interactions SER 101(H)...(N2) LIG1, SER 289(H)...(N5) LIG1, SER 94(H)...(N6) within LIG1, the length of hydrogen bonds are 1.75 Å -2 Å (with 1.75 Å showing the largest distribution), 2.5 Å -3 Å (with 2.75 Å the largest distribution) and 1.75 Å -2 Å (with 1.75 Å the largest distribution), respectively (Figures 7, left). In the LIG2 active side of enzyme (Figure 5), located on its surface, there are only three different amino acids: ASN 471, ASP 358, GLU 354.

In case of ligand docked at the enzyme surface, more precisely at LIG2, the interactions ligand - enzyme appear relatively late, during MD simulations, at about 30-40 ns (Figures 8, left) and all the formed HB are of medium and low strength (Figures 8, right). In addition, the formed bonds tend to disappear and re-appear during MD simulation (Figures 8, right).

In the interior of enzyme, at LIG1, there are two kinds of HB, formed between the enzyme and ligand: "amino acid (O) ... (H) ligand" and "amino acid (H) ... (N) ligand". In these cases, two amino groups, N_1H_2 and N_6H_2, are involved in the formation of hydrogen bonds (Figure 7). On the surface of enzyme, at LIG2, formation of HB of the type „amino acid NH (H) ... (N) ligand" involves the nitrogen atoms N_8 and N_6 of amino groups, while HB of the type „amino acid NH (H) ... (N) ligand" are provided by the nitrogen atoms of the amino groups N_6H_2 and N_2 (Figure 8)

Thus, the number and the type of amino groups NH_2 of ligand, involved in the formation of a complex with the enzyme, are two parameters that control the interaction ligand – enzyme. The stabilization of ligand – protein complex depends not only on the strength of the created HB but also on the number of each type of the created of hydrogen bonds.

CONCLUSIONS

The nature of interactions between Polyethylenimine and glucose oxidase 3QVR enzyme was studied by docking and Molecular dynamics techniques.

The docking procedure evidenced two places of major interaction of the protein with the polymer PEI: (LIG1), of -5.8 kcal/mol and (LIG2), of -4.5 kcal/mol, located inside the enzyme and on its surface, respectively. It was found that the hydrogen bonds HB, formed between enzyme and ligand, are shorter (i.e., stronger) inside of the protein compared to HB formed on the protein surface, which are, consequently, longer and weaker ones. The number and type of HB formed by the ligand with the active sites of the enzyme are important factors in the stabilization of the complex ligand – enzyme; also important are the dynamics of HB formation/breaking, within environmental conditions consideration.

ACKNOWLEDGEMENTS

R E F E R E N C E S

1. C. Sittiwet, M. Srisa-ard and Y. Baimark, *Polymer J.*, **2010**, *5*, 108.
2. T. Homma, T. Ichimura, M. Kondo, T. Kuwahara, M. Shimomura, *Eur. Polymer J.*, **2014**, *51*,130.
3. M. Shimomura, N. Kojima, K. Oshima, T. Yamauchi, S. Miyauchi, *Polymer J.*, **2001**, *33*, 629.
4. M. Poplawska, M. Bystrzejewski, I.P. Grudzinski, M.A. Cywinska, J. Ostapko, A. Cieszanowski, *CARBON*, **2014**, *74*, 180.
5. V.A. Karachevtsev, A.Y.U. Glamazda, E.S. Zarudnev, M.V. Karachevtsev, V.S. Leontiev, A.S. Linnik, O.S. Lytvyn, A.M. Plokhotnichenko, S.G. Stepanian, *Ukr. J. Phys.*, **2012**, *57*, 700.
6. A. Kasprzak, M. Popławska, M. Bystrzejewski, O. Łabędź, I.P. Grudziński, *Royal Soc. Chem. Adv.*, **2015**, *5*, 85556.
7. O. Trott, A.J. Olson, *J. Comp. Chem.*, **2010**, *31*, 455.
8. O. Trott, A.J. Olson, *J. Comput. Chem.*, **2010**, *31*, 455.
9. K. Dhananjayan, K. Kalathil, A. Sumathy, P. Sivanandy, *Der Pharma Chemica*, **2014**, *6 (2)*, 378.
10. R. Abagyan, M. Totrov, Curr. *Opin. Chem. Biol.*, **2001**, *5*, 375.
11. RCSB PDB, GOx - molecule of the month, May 2006.
12. C.I. Bayly, P. Cieplak, W.D. Cornell, P.A. Kollman, J*. Phys. Chem.*, **1993**, *97,* 10269.
13. S.A. Adelman, J.D. Doll, *J.Chem. Phys.*, **1976**, *64(6),* 2375.
14. J.P. Ryckaert, G. Ciccotti, H.J.C. Berendsen, *J. Comput. Phys.*, **1977**, *23(3),* 327.
15. W. Humphrey, A. Dalke and K. Schulten, *J. Molec. Graphics*, **1996**, *14*, 33.
16. B.R. Miller, T.D. McGee, J.M. Swails, N. Homeyer, H. Gohlke, A.E. Roitberg, *J. Chem. Theory. Comput.*, **2012**, *8 (9)*, 3314.
17. D.A. Case, T.A. Darden, T.E. III Cheatham, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo, R.C. Walker, W. Zhang, K.M. Merz, B. Roberts, B. Wang, S. Hayik, A. Roitberg, G. Seabra, I. Kolossváry, K.F. Wong, F. Paesani, J. Vanicek, J. Liu, X. Wu, S.R. Brozell, T. Steinbrecher, H. Gohlke, Q. Cai, X. Ye, J. Wang, M.J. Hsieh, G. Cui, D.R. Roe, D.H. Mathews, M.G. Seetin, C. Sagui, V. Babin, T. Luchko, S. Gusarov, A. Kovalenko, and P.A. Kollman. AMBER 11, University of California, San Francisco, **2010**.
18. G. Todde, S. Hovmoller, A. Laaksonen, F. Mocci, *Proteins*, **2014**, *82*, 353.