Poster E-4

Mutational Effects on Calbindin D9k Investigated by Molecular Dynamics Simulations

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Short Abstract: Protein activity is related to its tertiary structure and intermolecular interactions. Theoretical site-directed mutagenesis can be used to probe the structure and dynamics of proteins. Here, we apply molecular dynamics simulations to explore the effects of mutations on calbinding in an explicit aqueous environment.

Long Abstract:

The biological activity of proteins is related to its tertiary structure and intermolecular interactions. Moreover, the specificity of protein-ligand interaction plays an important role on these processes. Theoretical site-directed mutagenesis can be used to change these interactions so we can probe the structure and dynamics of proteins and its influence on ligand binding. Previous computational studies on calbindin were carried out using different protocols (force fields, treatment of the long range forces, ligand loaded states) and had shorter observation times (runs ranging from 100 to 400 ps) [1-4]. While some studies [2] relied on the wild-type protein structure, determined by X-ray crystallography [3,4] as the present work [5], Marchand and others used a mutant (P43G) structure obtained from NMR experiments1. Here, we apply molecular dynamics simulations to explore the effects of mutations on calbinding (3ICB) in an explicit aqueous environment.

We have performed 4 ns molecular dynamics (MD) simulations for the Calbindin D9k (CaB) with its calcium ligands loaded in the presence of explicit water and counter ions. Trajectories were generated via the GROMACS simulation software package [6-8]. Protein coordinates were taken from the Protein Data Bank [9] for the crystallographic structure of the CaB (pdb id 3ICB) [5]. Mutantational structures were built using the program Swiss PDB Viewer [10]. The mutants were obtained replacing the negatively charged residue glutamate (E) of the binding site by polar and neutral residues as glutamine (Q) and glycine (G), respectively. As a result, the systems investigated comprise the holo forms (protein with Ca2+ bound) of the following CaB molecules: the wild-type (W) and single mutants involving substitutions E17Q, E60Q, and E60G. In addition, we also studied the apo form of E60Q (protein without Ca2+). The proteins were modeled with GROMOS43a1 force field [11] and water molecules were represented by the SPC water model [12]. The production runs were carried out at 300 K for 4 ns and the long range interactions were treated using the particle-mesh Ewald summation scheme [13, 14].

The analysis of the simulations focuses on the structure and dynamics of calbindin molecules. Special attention is given to a particular mutation: E60Q. We have observed a fast conformational change of the residue glutamate 60 for the wild-type calbindin compared to the crystallographic structure, as observed before in a shorter MD simulation [4] and suggested by experiment [15]. This result emphasizes the importance of the interaction

between the ligand and the negatively charged carboxyl oxygen of residue 60 and points to some deficiencies of crystallographic structures for theoretical calculations. Our results also pointed out that the protein rigidity in binding site is greater for the wild-type protein. In addition, the length of side-chain of residue 60 was found important for the maintenance of the ligand in site I. Although this can help for maintaining the bound state, as observed by the similar behaviour with W and E60Q molecules, this could not be efficient to capture the ligand considering an apo form of protein due to the absent of a charged group, but this remains to be investigated. In general, our results are in agreement with experimental data on the mutants E17Q and E60Q [15] showing that neutralizing charged groups of site I leads to a substantial effect on site II, and that these structures are similar to the wild-type protein [16]. The structural and dynamical similarity of W and E60Q can explain the fact that these molecules exhibit very small differences on free energy of Gibbs [15]. In a forthcoming work we will compare the present results with electrostatic calculations.

References

[1] S. Marchand and B. Roux, PROTEINS: Structure, Function and Genetics, 33, 265 (1998).
[2] E. L. Mehler, J. N. Kushick and H. Weinstein, Molecular Simulation, 10, 309 (1993).
[3]J. Kördel and O. Teleman, J. Am. Chem. Soc., 114, 4934 (1992).
[4] P. Ahlström, O. Teleman, J. Kördel, S. Forsén and Bo Jönsson, Biochemistry, 28, 3205 (1989).
[5] D. M. Szebenyi and K. Moffat, J.Biol.Chem., 261, 8761 (1986).
[6] H. J. C. Berendsen, D. van der Spoel and R. van Drunen, Comp. Phys. Comm., 91, 43 (1995).
[7] E. Lindahl, B. Hess and D. van der Spoel, J. Mol. Model., 7, 306 (2001).
[8] D. van der Spoel, A. R. van Buuren, E. Apol, P. J. Meulenhoff, D. P. Tieleman, A. L. T. M. Sijbers, B. Hess, K. A. Feenstra, E. Lindahl, R. van Drunen and H. J. C. Berendsen, Gromacs User Manual version 3.1.1, Nijenborgh 4, 9747 AG Groningen, The Netherlands. Internet: www.gromacs.org (2002).
[9] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov and P.E. Bourne, Nucleic Acids Research, 28, 235 (2000).
[10] N. Guex and M. C. Peitsch, Electrophoresis, 18, 2714 (1997).
[11] W. R. Scott, P. H. Hünenberger, I. G. Tironi, A. E. Mark, S. R. Billeter, J. Fennen, A. E. Torda, T. Huber, P. Krüger and W. F. van Gunsteren, J. Phys. Chem. A, 103, 3596 (1999).
[12] H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren and J. Hermans, in: Intermolecular Forces (B. Pullman, ed.), Reidel, Dordrecht, 331 (1981).
[13] T. Darden, D. York, and L. G. Pedersen, J. Chem. Phys., 98, 10089 (1993).
[14] U. Essman, L. Perera, M. L. Berkowitz, T. Darden, H. Lee, and L. G. Pedersen, J. Chem. Phys., 103, 8577 (1995).
[15] S. R. Martin, S. Linse, C. Johansson, P. M. Bayley and S. Forsén, Biochemistry, 29, 4188 (1990).
[16] S. Linse, C. Johansson, P. Brodin, T. Grundström, T. Drakenberg and S. Forsén, Biochemistry, 30, 154 (1991).