THE INTERACTION FORCES OF THE BOVINE ALBUMIN TO DIFFERENT LIGANDS STUDIED BY AFM

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Molecular recognition involves different types of binding events between a particular type of the ligand and its macromolecular receptor. The understanding of the mechanism of this process is essential not only for the fundamental interest but also it can be used in pharmaceutics to design new drugs. One method, recently applied to such problems, is an atomic force microscopy (AFM). This technique enables measurements of molecular interaction forces very precisely (up to tenths of picoNewtons) giving the strength of the interaction occurring between single molecular complex (i.e. single ligand–receptor complex). The measurement of the unbinding forces as a function of loading rate (a linear change of a force with a time) can be used for quantitative characterization of the bond dissociation. This approach provides direct information about the dissociation rate related to bond lifetime and about the position of an energy barrier(s) present in a molecular energy landscape [1].

In this work, the AFM was applied to study the bond strengths and lifetimes of the typical antigen–antibody interaction. The studied system was the bovine serum albumin (BSA) and its monoclonal antibody (anti-BSA). Since BSA has also broad affinity to different types of small molecules, as fatty acids, the interaction with ethylene-diamino-tetraacetic acid (EDTA) was studied [2]. Verification of the specificity of the investigated complexes was performed by blocking the binding sites by adding a given type of molecules (either monoclonal antibody or EDTA). The obtained results showed that the interaction forces were larger for the BSA–EDTA than for the BSA–anti-BSA complexes. Also, the dynamic character of the unbinding of these complexes was completely different although the linear dependence of the most probable unbinding force as a function of the logarithm of loading rates was observed for both studied complexes: BSA–anti-BSA and BSA–EDTA. The energy landscape of the typical ligand–receptor complex (i.e. BSA–antiBSA) revealed only one energy barrier while for the BSA–EDTA complex two energy barriers were visible. These differences were attributed to the changes of the binding sites for all investigated molecules involved.

[1] E. Evans, Biophysical Chemistry **82** (1999) 83–97.

[2] J. Gryboś, G. Pyka-Fościak, K. Lebed, M. Lekka, Z. Stachura, J. Styczeń, Acta Physica Polonica **A 105** (2004) 501- 510.