Computational prediction of amino acid residues essential for ligand-binding selectivity of cytokinin receptors

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Cytokinins are essential plant hormones regulating plant growth and development. Natural cytokinins are a group of adenine derivatives having various side chains at N6 position of the purine moiety [1]. Several cytokinin receptors were identified in higher plants, differing in expression pattern and hormone preference [2]. The crystal structure of the ligand-binding module from typical cytokinin receptor *At*HK4 (CRE1/AHK4) from *Arabidopsis thaliana* was recently disclosed [3] allowing the comparison of different receptors by a computational approach. In the present study homology models for closely related receptors *At*HK2, *At*HK3 (from *Arabidopsis thaliana*), *Zm*HK1, *Zm*HK2 and *Zm*HK3 (from *Zea mays*) were built based on *At*HK4 template. Molecular docking of natural and synthetic cytokinins was performed, and an attempt was made to explain the structural basis of differential hormone selectivity.

Due to high homology of ligand-binding modules of cytokinin receptors, all the models built closely resemble the template. Altogether ligand binding sites consists of around 20 amino acids, half of them being conservative and other half to some extent variable. Both conservative and variable residues contact to adenine- or side chain moieties of natural cytokinins. Noticeable difference between the receptors is a presence of ~15 residue insertion between the positions 229 and 230 of *At*HK4 that resides both in *At*HK2/*Zm*HK3 and *At*HK3/*Zm*HK2 receptor ortholog pairs [4]. These insertions appear as non-structured loops in our models, but it is also possible that they form a β-hairpin. Despite they are located in the vicinity of the hormone binding site, they do not affect its conformation and cannot form contacts with the hormone molecule. *Trans*-zeatin (tZ) is the most interesting cytokinin as it is widespread natural hormone with high affinity to receptors and biological activity. *At*HK2, *At*HK4 and *Zm*HK3 have similar affinity for tZ whereas *At*HK3 and ZmHK1 bind it one order of magnitude stronger and weaker, respectively. The difference between *At*HK4 and

*Zm*HK1 is intriguing because the binding sites of these two ortholog receptors are very similar. Gly229 of *At*HK4 is deleted in *Zm*HK1, possibly affecting the conformation of the loop formed by residues 225-235. The most similar ligand specificity profile is observed for *At*HK2 and *At*HK4. The binding site composition is also very similar for these receptors: substitutions appear only at the periphery of the binding site (Tyr250His) or out of plane of adenine moiety (Leu251Ile, Ala322Thr) and affinity pattern must be defined by the delicate balance of these three substitutions.

On the basis of the molecular modelling and docking study we can conclude that the backbone conformation of the cytokinin binding site is rather similar in all tested receptors though deletion of some amino acids adjacent to binding site may influence site volume and shape. The difference in ligand specificity of receptors might be attributed to the difference in defined amino acids in binding sites. We have shown that a few noted amino acid substitutions lead to variations in volume and properties of cytokinin-binding pockets belonging to different receptors. To clarify the question of ligand-binding preferences of cytokinin receptors a series of receptor mutants was produced by site-directed mutagenesis and tested for hormone selectivity in binding assays. Binding energy calculation based on docking results for these mutants allowed us to rationalize experimental findings.

We acknowledge the financial support from the Program of Presidium of RAS and from grants of RFBR, projects 11-04-00614, 12-04-33282.

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