Revelation of the amino acid residues essential for ligand-binding selectivity of cytokinin receptors from arabidopsis and maize by computational approach.

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Cytokinin receptors differ in their ligand preferences. The molecular ground for differential ligand specificity between related cytokinin receptors could not be well understood before recent determination of crystal structure for typical receptor CRE/AHK4 CHASE domain containing the hormone binding site [1]. In the present study homology models for closely related receptors *At*HK2, *At*HK3 (from *Arabidopsis thaliana*), *Zm*HK1, *Zm*HK2, *Zm*HK3 (from *Zea mays*) were built based on CRE/AHK4 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 CHASE domains 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 residues insertion between the positions 229 and 230 of AtHK4 that resides both in *At*HK2/*Zm*HK3 and *At*HK3/*Zm*HK2 pairs [2]. 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 of cytokinins as it is the widespread natural hormone with one of the highest biological activity and affinity to receptors. Affinity of tZ is very similar for *At*HK2, *At*HK4 and *Zm*HK3, whereas it is one order of magnitude higher for *At*HK3 and *Zm*HK2, but one order of magnitude lower for ZmHK1. The latter finding is the most intriguing, because similarity between AtHK4 and ZmHK1 binding sites is substantial (all-atom RMSD between these structures is only 1.119 Å). 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 must be defined by the delicate balance of these three substitutions.

The ligand-binding similarity is rather unexpected, because in the pairs of receptors with more similar binding sites cytokinin affinity may differ in more than one order of magnitude.

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. Generally the difference in ligand specificity of receptors might be attributed to the difference in amino acid composition of binding sites. Such difference really exists, few noted amino acid substitutions lead to variations in volume and properties of cytokinin-binding pockets belonging to different receptors. To clarify the enigmatic question of ligand-binding preferences of cytokinin receptors few additional experiments in site-directed mutagenesis with consequent ligand-binding assay should be performed.

1. M. Hothorn, T. Dabi, J. Chory (2011) Structural basis for cytokinin recognition by *Arabidopsis thaliana* histidine kinase 4, *Nature Chem Biol*, **7**:766–768.

2. K. Yonekura-Sakakibara, M. Kojima, T. Yamaya, H. Sakakibara (2004) Molecular characterization of cytokininresponsive histidine kinases in maize. Differential ligand preferences and response to *cis*-zeatin, *Plant Physiol*, **134**:1654–1661.