## Structural Analysis of the Ligand Binding Domain of GluR0.

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**Introduction**: GluR0, a recently discovered prokaryotic glutamate receptor, exhibits unique ligand binding characteristics distinct from the AMPA, kainate and NMDA selective eukaryotic GluRs. To investigate the basis for this the ligand binding domain of GluR0 was expressed in E. *coli*, crystallized, and its structure solved by x-ray diffraction.

**Methods and Materials**: A four wavelength MAD data set was collected from a glutamate bound selenomethione derivative (20 - 2.0 Å) and native datasets for the glutamate (20 - 1.6 Å) and serine (20 - 1.9 Å) complexes.

**Results**: The fold of the ligand-binding core of GluR0 is similar to that of the eukaryotic AMPA receptor GluR2 and consists of a 2 alpha beta domains linked by two beta strands. GluR0 lacks one helix present in domain 2 and a loop present in domain 1 of GluR2. In both proteins the ligand interacts with domains 1 and 2 but the interactions are different for the prokaryotic and eukaryotic receptors. In GluR0 the bound glutamate molecule assumes an extended conformation with the gamma carboxyl group oriented towards and contacting domain 1. In contrast, in GluR2 the gamma carboxyl group points downwards and makes interactions with helix F in domain 2. In the serine complex a water molecule replaces one of the gamma carboxyl oxygens of glutamate and makes H-bonds with the serine hydroxyl group and a main chain amide nitrogen in domain 1. In GluR0 these ligand-mediated interactions between domains 1 and 2 are supplemented by numerous direct and water mediated H-bonds that may serve to stabilize the closed cleft conformation. Docking experiments show that AMPA and kainate cannot bind to GluR0 in the conformations found for GluR2 due to steric hindrance with residues in the ligand-binding pocket.

**Conclusions**: The mode of interaction of glutamate with GluR0, and the details of the folds in domains 1 and 2 more closely resemble the structure of the ligand bound periplasmic glutamine binding protein than GluR2 suggesting that GluR0 is a primitive signaling molecule that underwent additional functional and structural evolution to generate eukaryotic GluRs.

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