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A Photoswitchable Neurotransmitter Analogue Bound to Its Receptor

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Supporting Information

ABSTRACT: Incorporation of the azobenzene derivative *gluazo*, a synthetic photochromic ligand, into a kainate receptor allows for the optical control of neuronal activity. The crystal structure of *gluazo* bound to a dimeric GluK2 ligand-binding domain reveals one monomer in a closed conformation, occupied by *gluazo*, and the other in an open conformation, with a bound buffer molecule. The glutamate group of *gluazo* interacts like the natural glutamate ligand, while its *trans*-azobenzene moiety protrudes into a tunnel. This elongated cavity presumably cannot accommodate a *cis*-azobenzene, which explains the reversible activation of the receptor upon photoisomerization.

Biological photoreceptors are typically composed of a small light-absorbing molecule, the chromophore, which is bound covalently or noncovalently to a protein.[1,2] Accordingly, it is possible to convert proteins that are not inherently sensitive to light into photoreceptors by attaching synthetic photoswitches.[3]

Photoswitched tethered ligands (PTLs) and photochromic ligands (PCLs) have been developed for a variety of ion channels, including ionotropic glutamate receptors,[4] voltage-gated potassium and sodium channels,[5−7] transient receptor potential channels,[8] and pentameric ligand-gated ion channels.[9,10] They add a very useful functionality to these transmembrane proteins and allow the control of cellular functions with the temporal and spatial precision that is provided only by light.[6,11−13]

We have recently introduced PCLs that function as photochromic versions of the neurotransmitter glutamate.[7,14] One of these compounds, termed *gluazo* (Figure 1), proved to be a partial agonist of the kainate receptors GluK1 and GluK2 and was used to effectively control activity in dissociated hippocampal neurons and Purkinje neurons with light.[15]

Kainate receptors belong to the superfamily of ionotropic glutamate receptors and are tetrameric ion channels that contain four clamshell-like ligand-binding domains (LBDs). These adopt a closed conformation upon agonist binding, which is mechanically coupled to the opening of the intramembrane channel gate. Antagonists, by contrast, bind to the clamshell but do not allow sufficient closure to trigger gating.[16,17] A variety of soluble LBDs of kainate receptors, as well as related AMPA and NMDA receptors, have been crystallized, usually in complex with an agonist or antago-

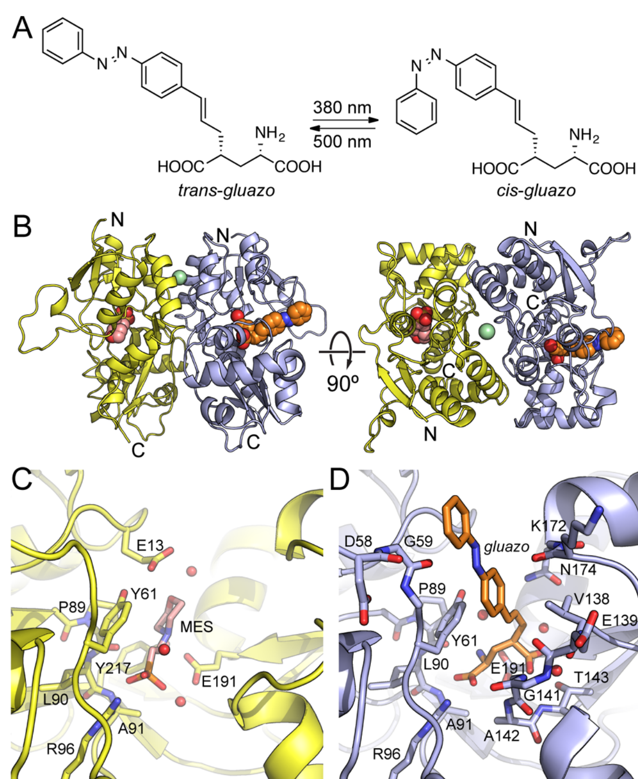

Figure 1. (A) Structures of *gluazo* in its *trans* and *cis* forms. (B) Cartoon representation of the GluK2-LBD homodimer crystal structure, perpendicular to (left) and along (right) its dyad axis. Monomer A (blue) in complex with *gluazo* (orange) adopts a closed conformation, while monomer B (yellow) shows the open conformation, which permits binding of a MES buffer molecule (pink). The central chloride ion is depicted as a green sphere. (C and D) Detailed overview of the GluK2-LBD binding pocket with bound MES and *gluazo* ligand, respectively. Residues and water molecules that form contacts with the ligands are shown as sticks and red spheres, respectively.

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nist.[18,19] In addition, the structure of an entire functional AMPA receptor tetramer in complex with the highly potent antagonist ZK-200775 has been elucidated by X-ray crystallography.[20]

Our design of *gluazo* was based on the structure−activity relationships of known kainate receptor agonists, such as LY-339434, which bears a lipophilic side chain attached to C4 of glutamate.[21] We anticipated that the azobenzene group of our PCL could substitute for this side chain, but only in its planar, stretched *trans* configuration (Figure 1). By contrast, the bent *cis* configuration would be incompatible with a closed (activating) LBD clamshell conformation. Hence, as the PCL toggles between an inactive *cis* and an active *trans* form, the channel protein could be gated in a light-dependent fashion. We now report the X-ray structure of *gluazo* bound to GluK2-LBD, which confirms our assumption. To the best of our knowledge, this is the first structure of a synthetic photoswitch in complex with a receptor protein.

GluK2-LBD expressed in *Escherichia coli* and purified was incubated with *gluazo* under dim-light conditions, yielding single crystals suitable for X-ray structural analysis. The structure of *gluazo* bound to GluK2-LBD, determined at 2.0 Å resolution, revealed a physiological LBD dimer[22] semi-occupied by the photochromic ligand (Figure 1B). Interestingly, one monomer (A) was found to be in the closed and the other (B) in the open conformation. Dimerization of the LBDs occurs via a quasi-symmetrical interface, similar to that of a symmetric glutamate-bound GluK2-LBD,[22] involving a buried surface area (BSA) of 933 Å^2, with 10 hydrogen bonds, four salt bridges, and a bound chloride ion at the dyad axis (Figure 1B).

The photochromic ligand *gluazo* is tightly bound to monomer A, between clamshell domains 1 and 2 (Figure 1D and Figure S1 of the Supporting Information); 77% (474 Å^2) of the solvent accessible surface of *gluazo* is buried in the complex, and 64% of the contacts are formed between the glutamate moiety of *gluazo* and GluK2-LBD, including eight hydrogen bonds (three of them water-mediated) and two salt bridges. In contrast, the larger azobenzene moiety of the *gluazo* ligand exclusively forms van der Waals (VdW) contacts and is engaged in hydrophobic interactions with an "exit tunnel". Notably, no polar contacts to the diazene unit (N=N), a well-known hydrogen bond acceptor, are observed.

Comparison of the GluK2-LBD·*gluazo* complex with previously described complexes of the kainate receptor LBD with the native ligand glutamate (GluK2-LBD·Glu)[23] or the natural product domoic acid (GluK2-LBD·domoate)[24] explains why *gluazo* functions as a partial agonist (Figure 2). The glutamate moiety of *gluazo* forms essentially the same contacts with GluK2-LBD as glutamate itself (Figure 2 and Figure S2 of the Supporting Information). Similar to the GluK2-LBD·Glu complex, the GluK2-LBD·*gluazo* structure contains five water molecules in the binding pocket in addition to the ligand. While three of these water molecules occupy the same position in both crystal structures, two of them are slightly displaced as a consequence of the larger opening angle between the two receptor domains in the GluK2-LBD·*gluazo* complex. In contrast to glutamate, however, which is almost completely buried in the GluK2-LBD·Glu structure (with 99% BSA), the much bulkier ligands *gluazo* and domoate cannot be fully accommodated and partially protrude from the binding pocket through an "exit tunnel" between domains 1 and 2 (Figure 2).

As a consequence, complexation of *gluazo* or domoate results in a more open conformation of the GluK2-LBD clamshell. The

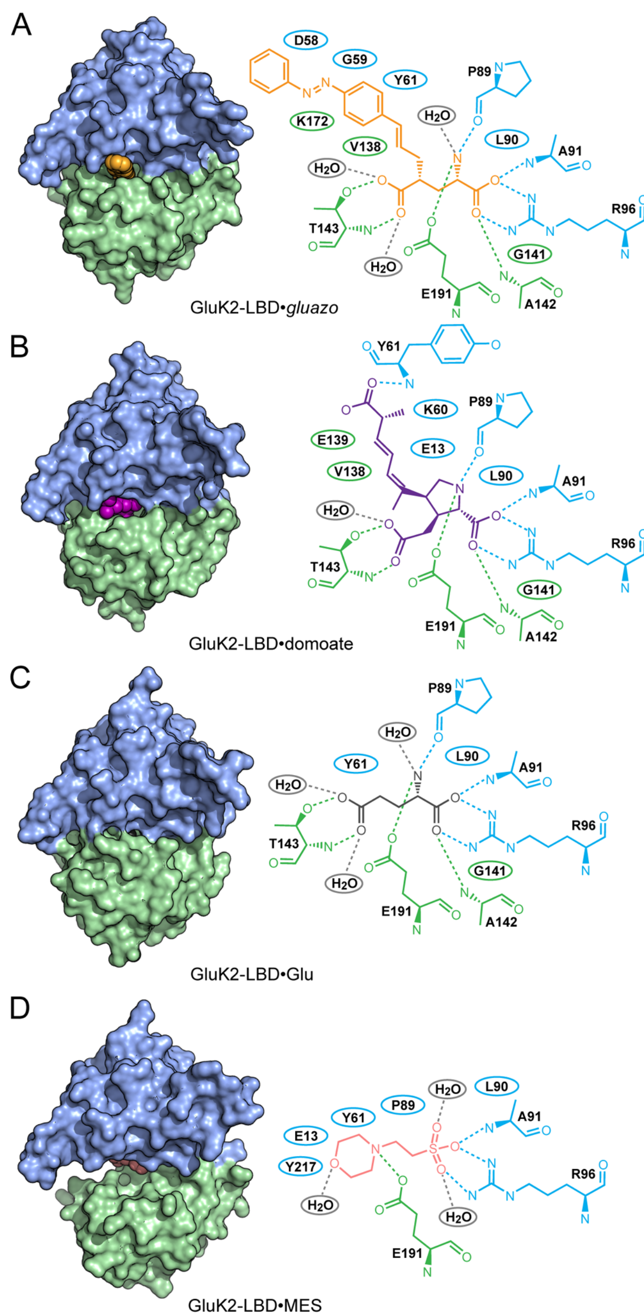

Figure 2. Surface representation of GluK2-LBD monomers (domain 1 colored blue and domain 2 colored green) in complex with (A) *gluazo* (orange), (B) domoate [purple; Protein Data Bank (PDB) entry 1YAE, chain A],[24] (C) glutamate (dark gray; PDB entry 3G3F, chain B),[23] and (D) MES (pink). Protein−ligand interactions are depicted next to the surface representations. GluK2-LBD residues forming hydrogen bonds with the ligand are shown as sticks, while amino acids that form van der Waals contacts are indicated as ellipses with the residue name and number.

respective angles between domains 1 and 2 of the GluK2-LBD·*gluazo* and GluK2-LBD·domoate complexes are increased by 8° and 13°, respectively (Figure S3 of the Supporting Information), showing that the azobenzene moiety of *trans*-*gluazo* can be better accommodated than the unsaturated side chain of domoate, which also forms a hydrogen bond at its side chain carboxylate. Because the degree of clamshell closure is

related to the extent of receptor activation, both *gluazo* and domoic acid are less efficacious than the full agonist glutamate.

Importantly, binding of *gluazo* to the basically closed form of the LBD clamshell appears to be only possible for the *trans* isomer of *gluazo*. Modeling shows that the azobenzene side chain in its *cis* form is not compatible with clamshell closure to an extent that activates the receptor (Figure S4 of the Supporting Information), which is in accordance with our electrophysiological results.

Unexpectedly, the other GluK2-LBD in the dimer (monomer B) accommodates 2-(*N*-morpholino)ethanesulfonic acid (MES), a component of the crystallization buffer (Figure 1C and Figure S1 of the Supporting Information). MES predominantly forms contacts with domain 1, partially overlapping with the binding site for glutamate. This clamshell adopts an open conformation similar to those found when antagonists are bound, while the asymmetric quaternary structure may also be favored by the crystal packing environment. The sulfonate group of MES is engaged in a salt bridge with Arg96 and a hydrogen bond with the main chain NH group of Ala91. The protonated morpholino nitrogen forms a salt bridge with Glu191 in receptor domain 2, which normally engages in a salt bridge with the amino group of glutamate (Figure 2). As such, MES mimics the α-amino/carboxylate moiety of glutamate. Apparently, *trans-gluazo* could not compete with MES, which was present at a much higher concentration in the crystallization buffer, for the second binding site in the clamshell dimer. It is conceivable that glutamate receptor antagonists could be developed on the basis of this previously unknown binding mode of MES.

In summary, we have determined the X-ray structure of a photoswitchable neurotransmitter bound to its receptor, which explains why *gluazo*, in its *trans* form, can act as an agonist of kainate receptors and loses activity upon photoisomerization to the *cis* form. As such, it facilitates the design of photochromic neurotransmitters and neuromodulators, in particular those interacting with clamshell-like LBDs. Finally, this structure represents the first case of a kainate receptor LBD dimer in which one clamshell resides in an open antagonist-bound conformation while the other adopts a closed, agonist-bound conformation.

■ ASSOCIATED CONTENT

Supporting Information

Atomic coordinates and structure factors have been deposited in the Protein Data Bank with the accession code 4H8I. Detailed experimental procedures, Table S1, and Figures S1–S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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