Flexibility of a Glutamate-Binding Domain

Robert E. Oswald[1,*]
[1]Department of Molecular Medicine, Cornell University, Ithaca, NY 14853, USA
*Correspondence: reo1@cornell.edu
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The molecular dynamics simulation of the binding domain of a glutamate receptor presented in this issue of *Structure* (Lau & Roux, 2007) provides insights into large-scale fluctuations of this protein that are supported by experiment and provide constraints on possible models for the function of the intact glutamate receptor.

Glutamate receptors are the major excitatory neurotransmitter receptors in the mammalian central nervous system and are involved in a range of neurological diseases (Parkinson's and Alzheimer's diseases, Huntington's chorea, and epilepsy) and in normal physiological processes, such as learning and memory. Drugs targeted to these receptors have enormous potential for treating neurological and psychiatric disorders. One of the important breakthroughs in the study of these receptors was the isolation of a soluble binding domain and the solution of the crystal structure (Armstrong et al., 1998). Although the binding domain represents an isolated portion of the intact protein, it binds agonists and antagonists with an affinity similar to the intact protein and has become an important model system for understanding the relationship between the structure and dynamics of the binding domain and the overall function of the protein. The crystal structures have provided a wealth of information on the structure of the binding site and have suggested clues to the coupling between binding, channel opening, and desensitization. However, the crystal structures are largely static and may, in some cases, be affected by crystal packing. Obviously, it is the motion of the protein that provides the initial signal that is transmitted to the ion channel and considerable effort has been made to understand the solution behavior of the binding domain using NMR spectroscopy (Ahmed et al., 2007), FTIR spectroscopy (Cheng et al., 2002), fluorescence spectroscopy (Abele et al., 2000), and small-angle X-ray scattering (Madden et al., 2005), as well as computational methods (Arinaminpathy et al., 2002; Mamonova et al., 2005). Each of these methods has provided new information highlighting different aspects of the structure and dynamics of this important protein. The protein consists of two lobes that are often assumed to move as rigid bodies, with the ligand-binding site between the two lobes. Upon binding of agonist, the lobes close and envelope the ligand, and a modest correlation has been observed between the degree of lobe closure in some crystal structures and the efficacy of an agonist. However, even the crystal structures suggest that the relative lobe orientation is not static. NMR (A.S. Maltsev and R.E.O., unpublished data), fluorescence (Ramanoudjame et al., 2006), and small-angle X-ray measurements (Madden et al., 2005) report on the average solution structure, which is in some cases, different from the crystal structure.

Lau and Roux (2007) used all-atom molecular dynamics in conjunction with umbrella sampling to compute the free energy landscape of the binding domain in the apo state, bound to an anagonist (DNQX) and bound to the natural ligand (glutamate). Two distances (ξ1 and ξ2; Figure 1) were defined between the lobes (defined by the center of mass of groups of 2–3 sequential residues in each lobe) that capture the degree of lobe closure and possible twists of one lobe relative to the other, and the starting coordinates for the umbrella sampling were positioned along ξ1 and ξ2. The overall results are completely consistent with previous experiments and simulations, but do add important details. That is, the free energy landscapes (described by the [ξ1,ξ2] order parameter) suggest a protein for which the relative orientation of the two lobes in the apo state is extremely flexible, somewhat less flexible in the antagonist-bound state, and relatively, but not completely, rigid in the full agonist-bound state. In fact, the results suggest that, in the apo state, the relative orientation of the two lobes can be considerably more open than that observed in the apo crystal structure (Figure 1). This hyperextension of the structure has been observed in the crystal structure of an antagonist-bound form (Kasper et al., 2006) but has not yet been observed directly in an apo crystal structure. However, as described in the paper, ensemble average scatter profiles calculated from the simulation trajectories match the small-angle X-ray scattering data (Madden et al., 2005) better than profiles computed from the crystal structures, suggesting that in solution these hyperextended forms may exist. These results are also consistent with ^{19}F NMR data (Ahmed et al., 2007), which show clearly that the apo form is very flexible. This is inferred from the dynamics of the four ^{19}F-labeled tryptophans, one of which is in the cleft between the two lobes. In the case of a similar antagonist (CNQX), the tryptophan in the cleft shows at least two different states, and for the glutamate-bound form, this tryptophan is relatively rigid.

Another interesting result of these studies is a suggestion as to the steps in the binding process, which potentially represent the reaction coordinate. Again, at least on a superficial level, the results are consistent with experiment. The idea that binding first occurs to lobe 1 (docking) followed by closure of lobes and interaction with

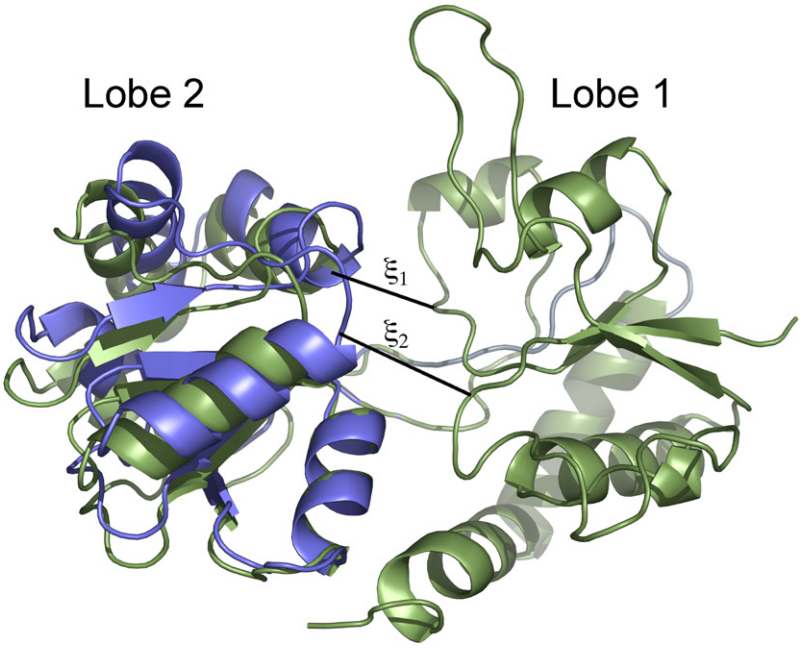

Figure 1. Structures of the S1S2 GluR2 Protein with the Two Lobes Opened by 16° (Blue) and 30° (Green) Relative to the Glutamate-Bound Structure, 1FTJ
This represents the range of openings represented by 91% of the conformational ensemble. The two distances shown (ξ_1 and ξ_2) were used to determine the two-dimensional order parameter.

lobe 2 (locking) has been proposed previously (Abele et al., 2000), but these simulations present a plausible pathway leading from the open state through two intermediates to a closed and fully bound state. Furthermore, the energetics of the process (stabilization of 9–12 kcal/mol) is similar to that obtained with isothermal titration calorimetry (approximately 8 kcal/mol, Madden et al., 2000).

The main interest in the motions of this protein is the idea that the closure of the lobes around the agonist forms the first step in the process leading to the opening of the ion channel. The receptor is formed from four subunits, each of which contains a copy of the S1S2 binding domain. Results from single channel recording at varying agonist concentrations have led to the notion that the conductance level of the channel may be correlated with the number of binding sites occupied, but it is not clear if simple closure of the lobes directly opens a conducting path in the ion channel or if lobe closure increases the probability that the channel will open, with other parts of the protein contributing essential roles (Oswald, 2004). Partial agonists, which in some cases bind with high affinity, preferentially populate lower conductance states. Extension of these studies to the free energy landscapes for partial agonists will be of considerable value for restraining possible models of channel activation.

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