NEUROPROTECTION

Structure-Based Functional Design of Chemical Ligands for AMPA-Subtype Glutamate Receptors

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Abstract

Ionotropic glutamate receptors (GluRs) function as an excitatory transmitter system in the human brain, particularly in learning and memory. Development of small molecules that are capable of selectively potentiating the ion channel activity of AMPA-subtype GluRs holds promise for potential new treatment of neurodegenerative diseases such as Alzheimer's. In working towards this goal, we obtained main-chain nuclear magnetic resonance (NMR) assignments of the extracellular ligand-binding domain of GluR2 that enables us to investigate receptor-ligand interactions in physiological conditions at atomic detail. With NMR structure-based methods, chemical compounds that can selectively modulate the ion chancel activity of GluR2 alone or synergistically with glutamate or kainate were identified. Our NMR structural analysis of GluR2 S1S2 further reveals that the regions of the receptor dimer interface exhibit distinct conformational dynamics, which we hypothesize to be linked to receptor functions in interactions with an agonist or antagonist. This coupling of ligand binding to receptor dimerization, gating, and desensitization may serve as an in vitro biophysical parameter to evaluate potential biological effects of the chemical ligands being developed here.

Index Entries: AMPA receptors; NMR; rational ligand design.

Introduction

Glutamate receptors (GluRs), a group of transmembrane proteins that function to channel calcium and sodium ions through cell membrane, play an important role in regulating human brain activities related to memory and learning (Seeburg, 1993; Hollmann and Heibemann, 1994; Morrison and Hof, 1997). Dysfunction of these protein receptors has been implicated in human brain neurodegenerative diseases, including Alzheimer's disease (Pellegrini-Giampietro et al., 1997; Lee et al., 1999). In an effort to understand mechanisms of action of GluRs, we have employed recently developed methods of structure-based ligand design with nuclear magnetic resonance (NMR) spectroscopy to develop selective small-molecular chemical compounds for modulating biological functions of AMPA ($\underline{\alpha}$-amino-3-hydroxy-5-$\underline{m}$ethy-4-isoxazole-propionic $\underline{a}$cid)-subtype GluRs (Burnashev et al., 1992; Hollmann and Heibemann, 1994; Kuusinen et al., 1995). These designed chemical ligands can be used as novel tools to study the mechanisms of action of the receptors and can also help further development of potential therapeutic agents that modulate receptor function in cells for new treatment of Alzheimer's disease.

Methods

High affinity and selectivity for biological targets are essential elements for the efficacy of potent therapeutic agents, which should also possess good bioavailability, metabolic stability, and low toxicity.

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Structure-based rational drug design offers great potential in drug discovery (Hajduk et al., 1999; Moore, 1999). Our approach of structure-based drug design utilizes NMR and a linked-fragment method to identify and refine chemical leads for development of specific ligands for therapeutic targets. Specifically, the chemical building blocks identified from NMR-based screening and optimized for binding to a protein selected as a target for treatment of a specific human disease. The resulting linked chemical compounds with high affinity and selectivity are then subject to detailed structure-based analysis of their interactions with the target protein using a combination of NMR and computational modeling (Weinstein and Osman, 1989). Refinement, chemical diversification through various chemical linkages, and selectivity enhancement are achieved at this stage.

Results and Discussion

Protein Sample Preparation and NMR Assignments

To improve the efficiency of recombinant protein sample preparation and to facilitate our search for chemical compounds, we have developed a new cDNA construct that produces soluble GluR2 protein from the bacteria expression system. Not only has this new construct enabled us to produce a large quantity of ^{15}N-labeled GluR2 protein for NMR screening of chemical compounds in this study, but it has also made it feasible for us to prepare uniformly ^{13}C/^{15}N-labeled and fully deuterated (^{2}H) protein for NMR assignments. These fully deuterated protein samples have aided us in obtaining NMR assignments for many amino acid residues located at the ligand binding sites of the protein, which often exhibit weaker NMR signals because of dynamics and possible conformational exchange. Particularly, because dipole-dipole relaxation pathways in amino acid side chains are minimized or eliminated by protein deuteration, the deuterated protein samples enabled us to acquire much better three-dimensional triple-resonance NMR spectra, which include HNCA, HN(CO)CA, HN(CA)CB, and HN(COCA)CB (Yamazaki et al., 1994). Furthermore, the improved relaxation properties of the fully deuterated protein have also enabled us to take an alternative approach with the use of three-dimensional ^{15}N-edited NOESY and TOCSY spectra collected with various mixing times. This NOE-based approach, which is complementary to that of triple-resonance backbone connectivity methods, takes advantages of sequential NOE patterns and known protein structural information from the crystallographic study. With these new NMR spectra and combined approaches, we have now obtained NMR assignments to >95% of the entire protein, which include amino acid residues at the key locations of the protein, particularly at the ligand binding sites.

Chemical Compound Screening and Lead Discovery With NMR

With the procedure that we have developed in this study, we have completed construction of a library of approx 15,000 chemical compounds that are largely obtained from commercial sources. Using a robotic system, we have prepared these chemical compounds in stock solutions in both single and mixture formats that are suitable for NMR screening. To facilitate our efforts in chemical compound screening, we have constructed a chemical compound database that stores various information from these chemical entities, including identification number, name, molecular weight, chemical structure, formula, and location coordinates. Currently, we are extending this chemical database to house experimental results that are being generated from chemical compound screening, as well as SAR (structure-activity relationship) studies by NMR and computational methods. This database is being operated with a web-based network system so that investigators within and between research laboratories who are involved in this project can readily retrieve their experimental results through this chemical/knowledge database, particularly for NMR-based chemical screening and SAR studies.

Guided with the almost complete main-chain resonance assignment of the protein, we have conducted NMR screening of chemical compounds by using the procedure developed in this study, as described previously. Out of several thousand chemical compounds screened, we have found several compounds that can bind specifically to the GluR2 S1S2 protein. More interestingly, some of the initial binding compounds can interact with the protein that is already bound to the known agonist AMPA. Currently, we are conducting detailed analysis of SARs of these initial lead compounds by studying interaction of their chemical analogs with GluR2 using NMR and computational methods. We are also making efforts to determine the structural basis of GluR2 recognition of these initial

binding compounds by identifying specific interacting amino acid residues of the protein. Our preliminary structural analysis data suggest that a few of the initial lead compounds interact with the protein in regions adjacent to the AMPA-binding site. Therefore, it is possible, conceivably, that linking an initial lead compound to AMPA could produce a combined ligand with much higher affinity and/or specificity.

Structural Analysis of GluR/Ligand Binding by NMR and Computatonal Methods

The structural analyses of GluR2 S1S2 in complex with agonists or antagonists have shown, surprisingly, that binding of GluR2 to different ligands takes place in almost exactly the same site in the protein and involves nearly the same set of amino acid residues. Recent studies show further that the ligand-binding cores of GluR2 can form dimers, and stabilization of the intradimer interface by either mutations or allosteric modulators reduces desensitization (Sun et al., 2002). Perturbations that destabilize the interface enhance desensitization. These studies suggest that receptor activation possibly involves conformational changes within each subunit that cause an increase in the separation of portions of the receptor that are linked to the ion channel, although the molecular basis for the action of an agonist or antagonist in gating and desensitization of GluRs as ion channels still remains elusive.

Our current NMR structural analysis reveals that the binding of some of our initial lead compounds to the GluR2 molecule causes the protein to undergo conformation exchange between monomer and dimer states. Notably, under the NMR condition, the free GluR2 protein exits predominantly in a monomeric conformation. Our NMR resonance assignment study showed recently that regions of the GluR2 molecule involved in subunit-subunit interactions in a dimer conformation, which has been implicated in the recent structural analysis to be important for the receptor activation, exhibit an unusual conformational exchange, as evidenced by significant line broadening observed in NMR spectra. Our NMR structural study further suggests that this conformational dynamics behavior of the protein might be linked to the molecular mechanism by which the protein interacts with its ligand, either an agonist or antagonist. On the basis of our NMR study, we postulate that the conformational dynamics of the receptor molecule, particularly in the regions involved in dimer interface, are directly dependent on whether the binding ligand is an agonist or antagonist. This newly discovered relationship of coupling of receptor ligand binding to gating serves as an excellent in vitro biophysical parameter to evaluate the potential effects of the chemical ligands that are being developed and optimized with our NMR and computational methods.

Electrophysiological Analysis of Lead Compounds on GluR2 Ion Channel Activity

To test and validate functional efficacy of the lead chemical compounds that are being developed in our NMR structural and computational modeling studies, we are also undertaking electrophysiological studies to evaluate the effects of these chemical compounds designed on the GluR ion channel activity. Particularly, we have expressed GluR2 subunits in *Xenopus* oocytes and measured the ion channel activity of GluR2 in response to the designed chemical compounds in reference to the responses produced by treatment of various agonists and antagonists. Our preliminary results indicate there is a good correlation between our in vitro NMR binding experiments and the in vivo electrophysiological recordings. We are also applying these functional assays to different GluR subunits. We expect that these electrophysiological ion channel activity assays, which are guided by our detailed NMR structural and computational analyses, will greatly enhance our ability in the optimization stage of the designed chemical ligands that show improved binding affinity and selectivity to different members of the GluR family.

Summary

The research progress that we have made in the past 12 mo in the important areas of our study (as summarized above), which is built on results obtained in the earlier stages of this project, has laid a solid foundation for the continuation of our NMR structure-based design for chemical ligands that bind to GluR2 S1S2 with selectivity. In the following months, we will be focusing our efforts on (1) characterization of the SARs of these initial lead compounds by studying the available chemical analogs by NMR; (2) determination of the structural basis of these lead compounds' interactions with the GluR2 protein by NMR and computational methods; (3) improvement and optimization of the initial lead compounds; and (4) evaluation of binding of the

improved chemical compounds to GluR2 and to other members of the GluR family by electrophysiology methods to improve ligand selectivity.

Acknowledgments

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