Solution X-ray Scattering Evidence for Agonist- and Antagonist-induced Modulation of Cleft Closure in a Glutamate Receptor Ligand-binding Domain*

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Dean R. Madden§§, Neali Armstrong%, Dmitri Svergun∥, Javier Pérez***‡‡, and Patrice Vachette**§§§

From the §Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755, the *Department of Biochemistry and Molecular Biophysics, Columbia University, New York, New York 10032, the ‡European Molecular Biology Laboratory, Hamburg Outstation, EMBL c/o Deutsches Elektronen-Synchrotron, Notkestrasse 85, 22603 Hamburg, Germany, and the **Laboratoire pour l’Utilisation du Rayonnement Electromagnetique (CNRS, UMR00130), Bâtiment 209d, B.P. 34, Université Paris-Sud, 91898 Orsay Cedex, France

Agonist-induced conformational changes in the ligand-binding domains (LBD) of glutamate receptor ion channels provide the driving force for molecular rearrangements that mediate channel opening and subsequent desensitization. The resulting regulated transmembrane ion fluxes form the basis for most excitatory neuronal signaling in the brain. Crystallographic analysis of the GluR2 LBD core has revealed a ligand-binding cleft located between two lobes. Channel antagonists stabilize an open cleft, whereas agonists stabilize a closed cleft. The crystal structure of the apo form is similar to the antagonist-bound, open state. To understand the conformational behavior of the LBD in the absence of crystal lattice constraints, and thus better to appreciate the thermodynamic constraints on ligand binding, we have undertaken a solution x-ray scattering study using two different constructs encoding either the core or an extended LBD. In agreement with the GluR2 crystal structures, the LBD is more compact in the presence of agonist than it is in the presence of antagonist. However, the time-averaged conformation of the ligand-free core in solution is intermediate between the open, antagonist-bound state and the closed, agonist-bound state, suggesting a conformational equilibrium. Addition of peptide moieties that connect the core domain to the other functional domains in each channel subunit appears to constrain the conformational equilibrium in favor of the open state.

The glutamate receptor ion channels (iGluR)1 are responsible for most excitatory synaptic signaling in the central nervous system (1). They are assembled as tetramers of subunits that share a modular design consisting of an N-terminal oligomerization domain, a ligand-binding domain (LBD), three transmembrane domains, a pore loop, and an intracellular C terminus (2, 3). The LBD of these receptors is structurally homologous to the bacterial periplasmic binding proteins (PBP) (4), for which spectroscopic, crystallographic, and solution-scattering analyses all established a Venus flytrap binding mechanism (5–8). The hallmark of this mechanism is a bilobate structure, in which the two lobes are separated by a binding cleft that is predominantly open in the empty state and closed in the ligand-bound state.

Data supporting a similar ligand-binding mechanism for the iGluR LBD have been obtained by spectroscopic (9, 10) and crystallographic (11) studies. The antagonist-bound LBD structure is open compared with agonist-bound states, and the extent of closure induced by a given agonist correlates broadly with its efficacy in activating the channel (11–14). Thus, the observed cleft closure appears to represent the driving force that is harnessed to open the channel gate. However, although the structures of the antagonist- and agonist-bound complexes appear to be generally well defined, it has been observed that the conformation of ligand-bound GluR2 LBD can differ as a function of lattice packing (11, 13, 15).

Furthermore, under physiological conditions, channel activation corresponds to the binding of agonist to a ligand-free binding site. As a result, understanding the molecular mechanism of iGluR activation will ultimately require a detailed understanding of the conformational status of the apo state. However, within the glutamate receptor family, different apo structures have been observed, suggesting that its conformational status may not be well defined. The crystal structure of the empty GluR2 LBD revealed an open cleft (11), whereas that of the bacterial GluR0 was closed (16). The ligand-free LBD of the metabotropic glutamate receptor mGluR1 has been crystallized in both open and closed conformations (17). Among the PBP, there are cases where the binding cleft is predominantly closed in the absence of ligand (18, 19). In other cases, multiple crystal

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† To whom correspondence may be addressed: Dartmouth Medical School, 7200 Vail Bldg., Hanover, NH 03755. Tel.: 603-650-1164; Fax: 603-650-1128; E-mail: drm0001@dartmouth.edu.
‡ To whom correspondence may be addressed: Synchrotron SOLEIL, L’Orme des Merisiers, Saint-Aubin-BP 48, 91192 Gif-sur-Yvette, Cedex, France.
§§ Present address: Institut de Biochimie et de Biophysique Moleculaire et Cellulaire, CNRS UMR 8619 Université de Paris-Sud, Bâtiment 430, 91405 Orsay Cedex, France.
∥∥ Present address: Laboratoire de Physico-Chimie et Biologie des Membranes, Université Paris-Sud, Orsay, France.
1 The abbreviations used are: iGluR, glutamate receptor ion channel; LBD, ligand-binding domain; PBP, periplasmic binding protein; SAXS, small-angle x-ray scattering; SEC, size-exclusion chromatography; AMPA, α-aminooxy-3-hydroxy-4-isoaxazole propionate; CNQX, 6-cyano-7-nitro-2,3-dihydroxyquinoxaline; DNQX, 6,7-dinitro-2,3-dihydroxyquinoxaline.

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forms of the ligand-free states have revealed a spectrum of open conformations (20, 21). As a result of these considerations, it would be advantageous to perform scattering measurements in solution, to assess the conformational behavior of the iGluR LBD in different functional states without the potential conformational restrictions imposed by crystal lattice formation. This is technically challenging, because in contrast to the PBP, where ligand binding can be associated with cleft closures of up to ~60°, full agonists induce a maximal 20–22° closure in the GluR2 LBD (11). In addition, earlier small-angle x-ray scattering (SAXS) experiments on a GluR4 LBD construct had been unable to detect changes between empty and glutamate-bound forms of the domain, presumably due to the small magnitude of the change (22). Nevertheless, calculations based on the crystallographic models suggest that the corresponding conformational changes should be detectable by SAXS measurements designed for optimal sensitivity (Table I).

Here, we present a collaborative series of SAXS experiments that establish the conformational behavior of the iGluR LBD in solution. A small but reproducible conformational change is observed between the open, antagonist bound state and the closed, agonist bound state, consistent with the crystal structures. The apo state, however, appears to sample a conformational equilibrium, whose balance is possibly influenced by components that lie outside the PBP-homologous core.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—The core GluR2 LBD (“S1S2J”) that had been used in crystallographic studies of the domain was expressed bacterially, purified, and refolded as described (11, 23). The full-length GluR4 LBD construct that had been used in previous solution scattering experiments (“S1S2-D”) was generated using the baculovirus expression system and purified as previously described (24). Finally, a full-length GluR2 LBD (“S1S2L”) was also refolded from bacterially expressed protein (23). S1S2L consisted of residues Ser383–Glu524 (S1), a GTDGN linker, and Glu526–Asn791 (S2), corresponding approximately to the GluR4 construct, but lacking N- and C-terminal FLAG and c-Myc tags, and with a shorter linker.

**Protein and Ligand Concentration**—Purified protein solutions were initially concentrated to 6–8 mg/ml in Centricon 10 concentrators (Amicon) and dialyzed extensively against SAXS buffer (50 mM Tris-HCl, 100 mM NaCl, pH 7.4 at 22 °C, 0.02% NaN₃) or SAXS-G buffer (SAXS-G buffer at 0.8 ml/min. Peak fractions were collected manually and used to prepare scattering samples without further dilution, at final concentrations between 3.5 and 4.3 mg/ml. Higher resolution scattering measurements were performed using undiluted stock solutions that had been concentrated to ~15 mg/ml.

Ligand stock solutions were prepared in 30 mM NaOH at the following concentrations: 7.5 mM a-amino-5-methyl-3-hydroxy-4-isoxazole propionate (AMPA), 15 mM 6-cyano-7-nitro-2,3-dihydroxyquinazoline (CNQX), 15 mM 6,7-dinitro-2,3-dihydroxyquinoline (DNQX), and 75 mM glutamate. All ligands were used at the same volume dilution (8:52), yielding final concentrations of 1, 2, and 10 mM, respectively. For measurements in the presence of ligand, an equivalent volume of 30 mM NaOH was added to the protein stock solution. Background scattering for each protein/ligand combination was measured for buffer supplemented with the corresponding volume of ligand stock solution. To minimize variability, calibrated positive displacement pipettes were used to prepare protein/ligand samples in parallel.

**Small-angle X-ray Scattering**—SAXS measurements were performed using the synchrotron radiation beam line D24 at the DORI storage ring of LURE. The instrument, the data acquisition system (27), and the evacuated measuring cell (28) have already been described. Measurements were performed in a quartz capillary (diameter ~1.5 mm) maintained at 15 °C (S1S2J, S1S2L, or S1S2-D not purified by SEC) and 5 °C (S1S2-D purified by SEC).

For S1S2J and S1S2L samples, two series of eight frames of 100 s each were recorded over the range of modulus of the scattering vector s from 0.0028 to 0.052 Å⁻¹ (s = 2 sin θ/λ, where θ is the scattering angle, λ is the wavelength of the radiation, λ = 1.488 Å). In the case of S1S2-D, eight frames of 200 s each were recorded over the s-range 0.0022–0.032 Å⁻¹ for the solution eluted from the SEC column, and over the s-range 0.0038–0.052 Å⁻¹ for concentrated solutions (29–37 mg/ml) not subject to SEC purification. Frames were visually inspected to check for x-ray damage, and those few exhibiting even a slight indication of damage were eliminated. Data were scaled to the transmitted intensity, before computing the average and standard deviation of each measurement and subtracting the scattering from the corresponding buffer.

Subsequently, patterns recorded using dilute and concentrated samples were scaled to concentration before splicing using the least-square regression for each protein/ligand combination was measured for buffer supplemented with the corresponding volume of ligand stock solution. To minimize variability, calibrated positive displacement pipettes were used to prepare protein/ligand samples in parallel.

Scattering intensities were computed from the atomic coordinates of the crystal structures of S1S2J either unliganded (pdb entry 1fto) or liganded (pdb entry 1ftl) (11) using the program CRYSOL, which takes into account the electron density in the border layer (32). Each Protein Data Bank entry contains results were essentially independent of the copy chosen. The calculated scattering profile is fitted to the experimental pattern using only two parameters: the radius of gyration Rg of the particle and the value of the intensity at the origin I0. This approximation is valid over a restricted s-range (typically 2πRg < 1.3).

The distance distribution function p(r) corresponds to the distribution of distances between any two volume elements within one particle. It has been determined using the indirect transform method as implemented in the program GNOM (31). This function provides an alternative estimate of the radius of gyration.

Scattering intensities were computed from the atomic coordinates of the crystal structures of S1S2J either unliganded (pdb entry 1fto) or bound to the agonist AMPA (pdb entry 1ftm) or to the antagonist DNQX (pdb entry 1fbl) (11) using the program CRYSOIL, which takes into account the hydration water by introducing a 3-Å-thick border layer surrounding the molecule (32). Each Protein Data Bank entry contains multiple copies of the S1S2J domain within the asymmetric unit. All curves presented here have been calculated using chain A, but the results were essentially independent of the copy chosen. The calculated scattering profile is fitted to the experimental pattern using only two adjustable parameters, the excluded volume of the particle V and the electron density in the border layer ρb, to minimize the discrepancy,

\[ \frac{1}{N-1} \sum_{i=1}^{N} \left( I(s_i) - \langle I(s_i) \rangle \right)^2 \]

where N is the number of experimental points, and \( I(s_i) \) and \( \langle I(s_i) \rangle \) denote the experimental scattering curve and its standard deviation, respectively. All fits were obtained with values of \( \Delta \rho = \rho_b - \rho_c \) in the range (0.03–0.04 e Å⁻³) corresponding to a density of about 1.10 times that of the bulk solvent and commonly observed with soluble proteins.
RESULTS AND DISCUSSION

Three iGluR LBD constructs were used in this work. The S1S2J construct corresponds to the refolded, bacterially expressed PBP-homologous core of the GluR2flop subunit used in most crystallographic studies (11, 33). The S1S2-D construct corresponds to the LBD of the GluR4flip subunit, expressed as a secreted protein in the baculovirus system and used in previous biophysical studies (9, 22, 24, 34). It contains the peptides that link the core domain to the adjacent transmembrane and oligomerization domains. The S1S2L construct is a bacterially expressed LBD from GluR2flop whose boundaries correspond closely to those of S1S2-D.

Controlling Protein Conformation and Oligomeric Status—Previous solution-scattering measurements compared the apo- and glutamate-bound forms of the S1S2-D domain (22). To test the possibility that conformational changes might have been obscured because the apo state spontaneously samples either fully or partially closed conformations, measurements were performed not only in the presence of agonist, but also in the presence of the antagonist DNQX, which has been shown crystallographically to stabilize the open conformation of the domain (11). To ensure maximal occupancy of the binding site in the presence of agonist, the higher affinity agonist AMPA was used instead of glutamate. Ligand concentrations were selected to ensure >99% saturation even at the high protein concentrations required for SAXS measurements. Finally, to enhance the sensitivity of the experiments to the very small changes expected, particular efforts were taken to reduce the scattering background and eliminate parasitic scatter.

To enhance protein homogeneity and stability, measurements were performed at controlled temperatures, and protein was stored prior to experiments at intermediate concentrations (6–8 mg/ml) and only concentrated to 15–40 mg/ml immediately prior to analysis. During the final concentration step, the bacterially expressed, full-length S1S2L domain of GluR2 exhibited precipitation. Initial inspection of Guinier plots obtained from S1S2L following centrifugation revealed significant residual aggregation, and further analysis of this construct was not pursued. Only slight evidence of higher molecular weight species was seen with the bacterially expressed core S1S2J construct or with the insect cell-derived S1S2-D construct. Nevertheless, to optimize sample homogeneity, both proteins were purified by preparative SEC. A small shoulder close to the dimer molecular weight was observed for S1S2J and S1S2-D. For S1S2-D, analytical SEC of the pooled monomeric peak revealed that its magnitude had been reduced from 2.6–3.2% to 1.7–1.8%. Solution-scattering experiments with the SEC-purified proteins did not reveal any evidence of residual heterogeneity.

The S1S2J Ligand-binding Domain Core—SAXS data clearly show that the S1S2J core construct is more compact in the presence of AMPA than in the presence of DNQX or in the absence of ligand. Clear differences were observed between the DNQX- and AMPA-bound scattering curves, with a non-random distribution of intensity differences extending up to three to five times estimated standard deviation on both sides of the

![Graphs and images showing experimental results](image-url)
solution than in the crystal lattice: the S1S2J-DNQX complex appears to be somewhat more open in distribution of reduced residuals (Fig. 2). As for the AMPA complex, the experimental data is more complex than for the agonist- or antagonist-bound tal structure for the DNQX complex (Table I).

Solution scattering is larger than that calculated from the crystal structure of the complex of S1S2J with kainate, a partition with incomplete cleft closure, similar to that seen in the crystallographic structure. However, the overall degree of compactness of the apo domain may be similar to that seen in the crystallographic structure. However, this does not necessarily mean that the apo domain is in good agreement with that calculated from the corresponding crystal structure (Table I), indicating that the apo curve (Fig. 2A) for the S1S2J-DNQX complex, calculated scattering intensities exhibit some differences from the experimental data with a χ value of 2.3 and a markedly non-random distribution of reduced residuals (Fig. 2C). If anything, the S1S2J-DNQX complex appears to be somewhat more open in solution than in the crystal lattice: the $R_g$ value determined by solution scattering is larger than that calculated from the crystal structure for the DNQX complex (Table I).

For the ligand-free domain, interpretation of the scattering data is more complex than for the agonist- or antagonist-bound states. As for the AMPA complex, the experimental $R_g$ of the apo domain is in good agreement with that calculated from the corresponding crystal structure (Table I), indicating that the overall degree of compactness of the apo domain may be similar to that seen in the crystallographic structure. However, unlike the AMPA complex, for which the reduced residuals are approximately random (Fig. 2A), those for the apo-S1S2J domain exhibit a non-random, oscillatory pattern, with a χ value of 2.0 (Fig. 2B), suggestive of a modest conformational difference. Of the two measures, the residual plot is more sensitive to changes than is the radius of gyration, which is an aggregate description of the electron density distribution of the domain and can therefore mask compensatory conformational changes.

Both the scattering curve (Fig. 1A) and the distance distribution function (Fig. 1D) for the apo form lie between the corresponding curves for the DNQX- and AMPA-bound states, suggesting that the time-averaged conformation of the apo state of S1S2J is intermediate between the antagonist- and agonist-bound forms. Based on the $P(r)$ curves, it is conceivable that the apo state represents a single, well defined conformation with incomplete cleft closure, similar to that seen in the crystal structure of the complex of S1S2J with kainate, a par-

### Table 1

| Sample         | $R_g$ Guinier | $R_g$ P(r) | $D_{max}$ |
|----------------|---------------|------------|-----------|
| S1S2J-DNQX     | 20.8 ± 0.3    | 20.8 ± 0.2 | 65 ± 3    |
| S1S2J apo      | 20.5 ± 0.2    | 20.4 ± 0.2 | 62 ± 3    |
| S1S2J-AMPA     | 20.0 ± 0.2    | 19.9 ± 0.1 | 59 ± 2    |
| Calculated    |               |            |           |
| S1S2J-DNQX     | 20.2          | 20.2       | 63 ± 2    |
| S1S2J apo      | 20.5          | 20.4       | 62 ± 2    |
| S1S2J-AMPA     | 19.8          | 19.6       | 61 ± 2    |

*Values calculated from crystal structures 1FTL chain A or B (DNQX), 1FTO chain A or B (apo), and 1FTM chain A, B, or C (AMPA) using CRY SOL (32).*

Baseline (Fig. 1B). Smaller differences following the same trend were also seen between the apo- and the AMPA-bound scattering curves (Fig. 1C). The associated conformational changes are reflected in the real-space distance-distribution function $P(r)$, which represents a one-dimensional analog of the crystallographic Patterson function. The $P(r)$ distribution obtained in the presence of AMPA shows a clear shift toward smaller intramolecular distances compared with the distributions observed in the apo- or DNQX-bound states (Fig. 1D). This change is also reflected in the values for the radius of gyration $R_g$ and the maximum intraparticle distance $D_{max}$ (Table I).

Overall, the conformational changes detected by solution scattering are consistent with those seen crystallographically, although the magnitude of the difference appears to be somewhat larger between the agonist- and antagonist-bound states in solution (Table I and Fig. 1E). Solution-scattering data for S1S2J in complex with AMPA provide an excellent match to those calculated based on the corresponding crystallographic structure, as modeled using the program CRY SOL (32), with a χ value of 1.4 and a practically flat distribution of reduced residuals (Fig. 2A). For the S1S2J-DNQX complex, calculated scattering intensities exhibit some differences from the experimental data with a χ value of 2.3 and a markedly non-random distribution of reduced residuals (Fig. 2C). If anything, the S1S2J-DNQX complex appears to be somewhat more open in solution than in the crystal lattice: the $R_g$ value determined by solution scattering is larger than that calculated from the crystal structure for the DNQX complex (Table I).

For the ligand-free domain, interpretation of the scattering data is more complex than for the agonist- or antagonist-bound states. As for the AMPA complex, the experimental $R_g$ of the apo domain is in good agreement with that calculated from the corresponding crystal structure (Table I), indicating that the overall degree of compactness of the apo domain may be similar to that seen in the crystallographic structure. However, unlike the AMPA complex, for which the reduced residuals are approximately random (Fig. 2A), those for the apo-S1S2J domain exhibit a non-random, oscillatory pattern, with a χ value of 2.0 (Fig. 2B), suggestive of a modest conformational difference. Of the two measures, the residual plot is more sensitive to changes than is the radius of gyration, which is an aggregate description of the electron density distribution of the domain and can therefore mask compensatory conformational changes.

Both the scattering curve (Fig. 1A) and the distance distribution function (Fig. 1D) for the apo form lie between the corresponding curves for the DNQX- and AMPA-bound states, suggesting that the time-averaged conformation of the apo state of S1S2J is intermediate between the antagonist- and agonist-bound forms. Based on the $P(r)$ curves, it is conceivable that the apo state represents a single, well defined conformation with incomplete cleft closure, similar to that seen in the crystal structure of the complex of S1S2J with kainate, a par-tial agonist (11). Indeed, the scattering curve calculated from the S1S2J-kainate complex provides a better fit to the SAXS apo curve (χ = 1.4) than does the calculated apo-S1S2J curve. However, the reduced residuals are still not randomly distrib-

Fig. 2. Comparison of experimental solution scattering patterns for the LBD core (red squares) with those calculated from corresponding crystal structures using the program CRY SOL (32) (blue solid line). Comparisons are shown for the AMPA-S1S2J complex (crystal structure, 1FTM) (A), the ligand-free S1S2J complex (crystal structure, 1FTO) (B), and the S1S2J-DNQX complex (crystal structure, 1FTL) (C). In A a 45:55 linear combination of experimental scattering curves of the DNQX and AMPA states is also shown (green). The reduced residuals of the least-squares fits (I$_{exp}$ - I$_{calc}$)/σ$_{exp}$) are shown on a linear scale for each plot beneath the x-axis. In B the reduced residuals of the fit to the linear combination are shown in green.
uted (data not shown), and the $R_g$ calculated for the kainate complex is 20.0 Å, indicating that the kainate model is too compact. In comparison to the single-curve fits, a 45:55 linear combination of the experimental curves for the DNQX and AMPA complexes yields an excellent fit to both the scattering curve ($\chi = 1.3$; Fig. 2B) and the $R_g$ (20.4 Å). This suggests that a time-averaged conformation is observed, with the apo state spontaneously sampling both open and closed conformations with comparable frequencies.

The observation of an open:closed conformational equilibrium for the apo state differs from the picture provided by the crystal structures of the GluR2 LBD, in which the apo form has the most widely open cleft, $-2.5^\circ$ more open than the DNQX complex (11). However, additional crystal structures are available for the apo-LBD from other glutamate receptors: taken together, the full set of models includes both open and closed conformations of the domain (11, 16, 17). Thus, the assumption of an essentially binary equilibrium between open and closed states provides the most parsimonious explanation of the observed SAXS data that is also consistent with all crystallographic evidence. It is also consistent with the energetics of lattice formation, which are most likely to favor crystallization of conformations already existing in solution rather than distorting a unique, stable intermediate conformation, forcing the two lobes together or apart. Finally, the hypothesis that the apo state samples the closed state frequently enough to contribute significantly to the SAXS pattern of the solution is also consistent with molecular dynamics calculations in the absence of agonist (35).

However, the existence of more complex conformational equilibria, analogous to the multiple open conformations seen for the allose- and ribose-binding proteins (20, 21), cannot be excluded. Indeed, some plasticity of the open state is implied by the observation of crystallographically independent open apo states whose degree of cleft closure differs by $-4-5^\circ$ (11) and by modeling studies suggesting a greater conformational flexibility of the GluR2 apo domain compared with the agonist-bound state (35). There have been suggestions that changes in intradomain and interdomain mobility upon agonist binding may also be involved in channel gating (10, 35). However, we still do not understand the extent to which the apparently greater degree of conformational flexibility in the apo state of the ligand-binding domain is related to channel gating and desensitization, nor the extent and frequency with which the ligand-free binding domain may undergo spontaneous closure in the context of an intact receptor. Whatever the interpretation of the data for the apo form of the core LBD, it is clearly different from the static description of a single, wide-open form seen in the crystal.

**Full-length Ligand-binding Domain**—Analogous scattering experiments were performed on S1S2-D. Preliminary measurements on protein that had not been purified by size-exclusion chromatography revealed $R_g$ values of $-29$ Å for both the DNQX and AMPA complexes; however, no clear difference could be observed (data not shown). All further experiments were therefore performed with the SEC-purified material. In addition, we decided to increase the redundancy of the measurements to provide internal consistency checks. In addition to DNQX and AMPA, experiments were performed on complexes with the antagonist CNQX and the full agonist glutamate. Furthermore, two datasets were collected for each complex, using protein from two independent protein preparations.

Under these conditions, a clear and reproducible difference could be detected between the radii of gyration of the antagonist-bound complexes on the one hand and the agonist-bound complexes on the other (Table II). This difference is also clearly reflected in the distance-distribution functions, whose peak is shifted to smaller interparticle distances in the presence of agonists (Fig. 3A). Similar results were obtained with the second dataset (data not shown).

![Image A](image1.png)

**Table II**

| Sample  | $R_g$ Guiner ($\AA$) | $R_g$ $P(r)$ ($\AA$) | $R_{max}$ | $D_{max}$ ($\AA$) |
|---------|---------------------|----------------------|-----------|------------------|
| CNQX    | $28.7 \pm 0.3$      | $29.8 \pm 0.3$       | $28.9$    | $115 \pm 5$     |
| DNQX    | $29.0 \pm 0.3$      | $30.0 \pm 0.3$       | $29.2$    | $110 \pm 5$     |
| Apo     | $28.5 \pm 0.3$      | $30.1 \pm 0.3$       | $28.6$    | $112 \pm 5$     |
| Glu     | $28.0 \pm 0.3$      | $29.0 \pm 0.3$       | $27.5$    | $104 \pm 5$     |
| AMPA    | $28.2 \pm 0.3$      | $28.9 \pm 0.3$       | $27.7$    | $106 \pm 5$     |

A comparison of the distance-distribution function of the apo-S1S2-D domain with those of the antagonist-bound proteins revealed only very small differences (Fig. 3B). Changes are concentrated along the falling edge (35 Å $\leq r \leq 60$ Å) but are only slightly greater than the variability seen comparing three independent $P(r)$ curves for the S1S2-D-glutamate complex.

Fig. 3. Solution conformational behavior of the extended GluR4 LBD S1S2-D. Normalized distance-distribution curves are shown for agonist- versus antagonist-bound conformations (A), and for the apo state and the antagonist-bound conformations (B). In A and B curves are color-coded according to the ligand-bound status of the complex: apo (black), CNQX (green), DNQX (blue), glutamate (red), AMPA (yellow). Insets highlight the region around the peak of each curve ($R_{max}$).
plex (not shown). As calculated from the Guinier plots, the $R_g$ value for the apo state appears to be slightly smaller than that of the antagonists, as is the radius corresponding to maximum $P(r) (R_{max})$, but the values derived from the distance distribution function show no significant difference (Table II). Thus, the difference between the apo- and antagonist-bound conformations, if any, appears to be smaller for S1S2-D than for the S1S2J core domain. The reason for this discrepancy with the core domain is not clear, but it suggests that the additional moieties included in the extended construct may preferentially stabilize the open state. Relative stabilization of the open state of the S1S2-D construct is supported by the kinetics of agonist docking, which indicate that the majority of ligand-free molecules are in an open, binding-competent conformation (9).

Compared with the experimental and calculated $R_g$ values for the core domain, the experimental values obtained for S1S2-D are significantly larger ($28–30$ Å versus $20–21$ Å), consistent with the presence of additional protein and carbohydrate moieties on the full-length domain (Tables I and II). The maximum intraparticle distance ($D_{max}$) is also significantly greater ($105–115$ Å versus $60–65$ Å for S1S2J). These differences are larger than expected based solely on the increase in molecular mass (42 versus 29 kDa), which would yield $R_g$ values of $26$ Å and $D_{max}$ values of $74$ Å for the extended construct, assuming similar axial ratios. The even larger values observed for S1S2-D thus most likely reflect the extended construct, assuming similar axial ratios. The fact that it is significantly more elongated than the core domain, implying that the additional moieties of the full-length domain must be clustered near the ends of the core domain, as had been indicated by previous measurements on S1S2-D (22).

Despite this confirmation of the elongated nature of S1S2-D, the $R_g$ values determined here are smaller than those measured previously (22): $28–30$ Å versus $31–34$ Å. It is therefore possible that the earlier SAXS experiments were affected by residual higher molecular weight species, whose contribution to scattering may have helped to obscure the small conformational change associated with agonist binding.

**CONCLUSIONS**

These data show that both the core and extended iGluR LBD constructs undergo a transition in solution from a more open, antagonist-bound conformation to a more compact, agonist-bound conformation corresponding broadly to that seen in the crystallographic structures of the core domain (11). As a result, all structural and biological data are now in agreement that the iGluR LBD undergoes a cleft closure stabilized by agonist binding and that the magnitude of this closure is smaller than those seen for many PBP. Together with recent evidence that the construct retains a high affinity binding site capable of ligand exchange and at least limited ($\sim 3^\circ$) cleft closure within the crystal lattice (15), these data underline the overall functional equivalence of the crystal and solution states of S1S2J in the presence of ligand. In the absence of ligand, it appears that the core domain samples a conformational equilibrium, including open and closed states, and that the equilibrium may be biased toward the open state by the addition of peptides that anchor the S1S2J core to the other domains in a complete iGluR subunit.

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