Amino Acids (2013) 44:1247–1251
DOI 10.1007/s00726-013-1461-9

INVITED REVIEW

Interaction proteomics of the AMPA receptor: towards identification of receptor sub-complexes

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Received: 3 January 2013 / Accepted: 5 January 2013 / Published online: 24 January 2013

Abstract AMPA receptors (AMPAR) are the main ligand-gated ion channels responsible for the fast excitatory synaptic transmission in the mammalian brain. Whereas a number of proteins that interact with AMPAR are known to be involved in the trafficking and localization of the receptor and/or the regulation of receptor channel properties, the protein composition of the AMPAR supra-complexes are largely unclear. Recent interaction proteomics report the presence of up to 34 proteins as high-confidence constituents of the AMPAR. It was proposed that the inner core of the receptor complex consists of the GluA tetramer and four auxiliary proteins comprising transmembrane AMPA receptor regulatory proteins and/or cornichons. The other AMPAR interactors, present in lower amount, may form the outer shell of the AMPAR with a range in size and variability.

Keywords Synapse · Excitatory neurotransmission · AMPA receptor · Protein complex · Proteomics

Abbreviations

AMPAR	AMPA receptor
BN-PAGE	Blue-native polyacrylamide gel electrophoresis
ESI	Electrospray
IP	Immuno-precipitation
MALDI	Matrix-assisted laser desorption ionization
MS	Mass spectrometry
TARP	Transmembrane AMPA receptor regulatory protein

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In the mammalian central nervous system the major fast excitatory synaptic transmission is mediated by ionotropic glutamate receptors of the AMPA subtype. AMPA receptor (AMPAR) form a tetrameric assembly, usually existing as double-dimer of the combination of four pore-forming subunits GluA 1–4, where GluA 1/2 and GluA 2/3 are the predominant forms (Lu et al. 2009). During synaptic transmission, the presynaptically released glutamate diffuses through the synaptic cleft and binds to the AMPAR located in the postsynaptic membrane. This leads to AMPAR channel opening that allows the influx of cations, especially sodium ions, which drives postsynaptic depolarization. Consequently, the abundance of AMPAR at the synapse determines synaptic efficacy. This may depend on the AMPAR (or an alternative fast, ionotropic glutamate receptor such as kainate receptor) trafficking from the extrasynaptic reserve pool (Granger et al. 2012).

Over the past decades a number of cytosolic proteins have been implicated in the insertion or removal of AMPARs from the synapse. These processes are often dynamically regulated in a neuronal activity-dependent manner, which underlies synaptic plasticity (Anggono and Huganir 2012) that is believed to be the basis of learning and memory. More recently, several membrane proteins, the presumably auxiliary proteins of AMPAR, have been shown to modulate AMPAR channel properties including current amplitude, desensitization and deactivation rates, resensitization, as well as trafficking of the AMPAR

(Jackson and Nicoll 2011; Straub and Tomita 2012). The precise protein constituents and the spatio-temporal pattern of the AMPAR complexes, however, are still largely unknown.

Recent interaction proteomics studies reported an overlapping list of proteins that interact stably with the AMPAR. The success of these analyses is rooted in the advancement of proteomics technology, in particular the increase in mass accuracy and sensitivity of mass spectrometry (MS). Low abundant proteins that were missed in studies only just a few years ago can now be revealed and quantified. In this mini-review we will discuss how the recent articles (Schwenk et al. 2009, 2012; Shanks et al. 2012; von Engelhardt et al. 2010) provide novel insights into the identity of AMPAR complexes.

As with other interaction proteomics experiments, Schwenk et al. 2009, 2012, Shanks et al. 2012, and von Engelhardt et al. 2010 immuno-precipitated (IP) the AMPAR complex, from detergent-extracted tissues. The samples were trypsin digested and the resulting peptides were analyzed by liquid chromatography–MS. Whereas the general workflows were similar, there are important differences between these studies (Table 1). A critical issue is the choice of the buffer constituents that should maintain, as much as possible, the integrity of the protein complex during (1) extraction of the tissue while maximizing extraction efficiency, and (2) subsequent relatively long incubation with the antibody for immuno-isolation before capturing the protein complex for proteomics analysis. The commonly used detergents, CHAPS and Triton-X100, were used by Shanks et al. (2012) and von Engelhardt et al. (2010), respectively, for tissue extraction. Schwenk et al. (2012) examined the usefulness of different detergents. They concluded that Triton X-100 causes loss of some of the protein complex constituents. These proteins can be readily detected when they used the buffers they developed, namely the ComplexioLyte buffers with mild or intermediate stringency. There are different types of ComplexioLyte buffers, each of which have differential effects on the recovery of the AMPAR interactors. For example, the novel AMPAR interactors ABHD-6 and CPT-1 (Table 2) were identified at much higher levels when the mild buffer ComplexioLyte CL-47 was used compared to that of stringent buffer CL-91. On the other hand, CL-91 solubilized 100 % of the total pool of the AMPAR, whereas CL-47 solubilized a moderate 40 %. The authors classified three protein categories (Table 2) based on their stability of interaction with AMPAR under low, intermediate and stringency ComplexioLyte conditions. The nature of the chemical composition of the ComplexioLytes was not disclosed.

The studies identified overlapping set of proteins, in particular the four AMPAR subunits GluA 1–4, the transmembrane AMPA receptor regulatory proteins (TARPs), and to a lesser extent CNIHs, CKAMPs (shisa), PRRT1 and 2, DLGs and Noelins. The different number of proteins identified may have been caused by technological and/or biological factors. (1) Earlier proteomics studies used MS of lower sensitivity and might have missed proteins of lower abundance (Schwenk et al. 2009 vs. 2012). (2) The use of different types of mass spectrometric ionization method [electrospray (ESI) vs. matrix-assisted laser desorption (MALDI)] may bias against the detection of some small proteins. For example, a number of tryptic peptides generated from CNIHs could be detected in the ESI-MS because the larger fragments with multiple charged states fall within the detection mass range, whereas only two peptides fall within the MALDI-MS detection mass range and therefore might have been missed in the MS analysis. (3) Schwenk et al. (2009, 2012) used the whole brain for analysis. Some of these AMPAR interacting proteins may not be present in the forebrain and therefore would not have been detected in the study of von Engelhardt et al. (2010). (4) Shanks et al. (2012) used brain from juvenile P15 rat as input. As GluA show developmental shift of subunits composition, it is expected that the AMPAR complexes of juvenile and adult animals have certain differences reflecting the developmental stages of the synapses.

The most recent study (Schwenk et al. 2012) reported the identification of more than 1,000 proteins across the IP experiments. An important issue is to distinguish true AMPAR interacting proteins in such a large number of IP-ed proteins. In a typical IP/MS experiments, hundreds of proteins could be characterized, probably majority of them are false-positive. Proper negative controls are needed to exclude these “contaminants” (see discussion in Li et al. 2012). Both Schwenk and von Engelhardt used specific AMPAR subunit knockout mice as negative control. Furthermore, Schwenk et al. (2012) used ten different antibodies for IPs, namely three against GluA1, three against GluA2, one against GluA2/3, one against GluA3 and two against GluA4. Together, merely 34 proteins were scored as high-confidence constituents of native AMPAR in the rodent brain (Table 2). Some of the interactions were further confirmed by reverse IPs. On the other hand, Shanks et al. (2012) used IgG as negative control for the IPs. This negative control alone may not be stringent enough to exclude the false positives, and many of the potential AMPAR interactors remain to be validated.

Whereas these studies provide a comprehensive list of AMPAR interactors, likely additional AMPAR interacting proteins exist. In the past decades, the use of yeast two-hybrid approach with the intracellular domains of AMPAR as bait led to the identification of a number of AMPAR interacting proteins including GRIP, PICK1, NSF, protein

Table 1 The experimental approaches of the four interaction proteomics studies

	Schwenk et al. (2009)	von Engelhardt et al. (2010)	Schwenk et al. (2012)	Shanks et al. (2012)
MS	ESI LTQ-FT MS (Thermo)	MALDI TofTof MS (4700 proteomics analyzer, AB-Sciex)	ESI LTQ-FT ultra MS (Thermo)	ESI LTQ and ESI LTQ-Orbitrap (Thermo)
Detergent	ComplexioLyte 48 and 91	Triton X-100	ComplexioLyte 47 and 91	CHAPS
Input	Plasma membrane-enriched protein fractions from total rat brain (P30)	P2 fraction from adult mouse forebrain	Plasma membrane-enriched protein fractions from total rat/mouse brain	Membrane fractions from total brain of juvenile rats (P15) or human brain cortex
Digestion	In-gel digestion from SDS-PAGE	In-solution digestion	In-gel digestion from SDS-PAGE	In-solution digestion
Control	IgG	GluR-A knockout mouse	IgG; GluR-A knockout mouse; GluR-B knockout mouse	IgG

Table 2 The AMPA receptor interacting proteins

Schwenk et al. (2009)	von Engelhardt et al. (2010)	Schwenk et al. (2012)	Shanks et al. (2012)
GluA1; GluA2; GluA3; GluA4	GluA1; GluA2; GluA3; GluA4	GluA1; GluA2; GluA3; GluA4	GluA1; GluA2; GluA3; GluA4
TARP γ2; TARPγ3; TARPγ4; TARPγ7; TARPγ8; TARP γ5	TARP γ2; TARP γ8	TARP γ2[a]; TARPγ3[a]; TARPγ4[a]; TARPγ7[a]; TARPγ8[b]	TARP γ2; TARPγ3; TARPγ4; TARPγ7; TARPγ8; TARP γ5
CNIH-2; CNIH-3		CNIH-2[c]; CNIH-3[c]	CNIH-2; CNIH-3
	CKAMP44	CKAMP44[a]; CKAMP52[b]	CKAMP44; CKAMP52
		C9orf4[a]	
	Noelin 1 (Pancortin)	Noelin 1[c]; Noelin 2[c]; Noelin 3[c]	Noelin 1; Noelin 3
		MAGUKp55-2	
		DLG 1[b]; DLG 3[b]; DLG 4[a]	DLG 1; DLG 2; DLG 3; DLG 4
	PRRT1 (NG5)	PRRT 1[c]; PRRT 2[c]	PRRT 1; PRRT 2
		Neuritin[c]	
		GSG1-l[a]	GSG1-l
		Brorin	
		Brorin-2 l	
		LRRT-4	
		PORCN[a]	
		PIP-PP SAC1[b]	
		ABHD-6; ABHD-12	
		CPT-1	
		Rap-2b	

Shanks et al. (2012) used normal rabbit IgG for parallel purification as negative control, which may not be stringent enough to define true interactors (see discussion in Li et al. 2012). For comparison the proteins shown here are those overlapped with Schwenk's study (Schwenk et al. 2012). The three protein categories based on their stability of interaction with AMPAR under low, intermediate and stringency detergent conditions (Schwenk et al. 2012)

[a] Interactions that are markedly reduced under conditions of high stringency

[b] Interactions mainly preserved at low stringency conditions

[c] Interactions that are largely unaffected by intermediate and high stringency conditions

4.1 and SAP97. Most of these proteins are not detected by interaction proteomics probably due to a low strength or transient nature of the interaction (see Anggono and Huganir 2012), which might cause proteins to be lost during the IP procedure. Interestingly, there are recent reports of stable AMPAR-associated proteins that had not been detected in these interaction proteomics experiments. The failure of their detection may be due to their low expression levels/low amount of the total protein population engaged in AMPAR interaction or their highly

selected brain region-specific or developmental expression patterns. For example, Syndig1 known to be involved in excitatory synapse development (Kalashnikova et al. 2010) was not found in any AMPAR interaction proteomics experiments.

Given the multitude of AMPAR interactors, it is likely that specific AMPAR complexes exist that are part of distinct physiological processes, e.g., trafficking, anchoring and AMPAR modulation at the postsynapse. A crucial question that has remained unanswered is how these complexes are organized, and more specifically which AMPAR partners are acting together. Schwenk et al. (2012) employed blue-native polyacrylamide gel electrophoresis (BN-PAGE) to dissect molecularly the AMPA receptor complexes. BN-PAGE separates native protein complexes according to their mass differences, which are subsequently detected and quantified by immuno-staining or MS. BN-PAGE of extracts of rat brain membranes followed by western blotting analysis with antibodies against AMPAR subunits revealed AMPAR complexes with apparent molecular mass of about 0.6–1.0 MDa (Schwenk et al. 2012). This is substantially heavier than the GluA tetramers of about 0.5 MDa. The mass range of 0.4 MDa in the apparent molecular masses of 0.6–1.0 MDa indicates the existence of multiple AMPAR complexes with different or overlapping protein constituents.

An antibody shift assay is often used to verify the co-existence of proteins in a complex. The binding of specific antibodies to a target protein causes a shift of the whole target protein complex on the BN-PAGE by the added mass of the antibody. A corresponding mass shift of a protein complex, therefore, is indicative of the presence of the target protein in the complex. Using antibodies against TARPγ2/3/8 in shift assays, Schwenk et al. (2012) demonstrated that all the solubilized AMPAR contain at least one of the TARPs. TARPγ8 and CNIHs co-shifted indicating that they associate in the same AMPAR complex. On the other hand, most of the TARPγ2 and γ3 proteins are assembled into a distinct sub-population of the AMPAR complexes. Based on this observation it is suggested that the inner core of the AMPAR complex consists of the GluA tetramer and four auxiliary subunits CNIHs and/or TARPs. The remaining AMPAR interactors probably exist at lower level in the AMAPR complex. For many of the proteins found associated with the AMPAR (Table 2), the specific stoichiometry and conditional presence with AMPAR remains unclear. Schwenk et al. (2012) classified these as "outer core" constituents.

Taken together, Schwenk et al. (2012) postulated a model of the AMPAR interactome which contains a common inner core comprising AMPAR and TARPs and/or CNIHs, and a variable peripheral extension with different proteins. Together, these promote the formation of AMPAR which range in size and in molecular composition. Could this model alone encompass all the proteins listed in Table 2? Probably not. The membrane fraction that was the input of the proteomics analysis is of heterogeneous nature and includes the post-synaptic density as well as membranes from other organelles. It is not unlikely that sub-populations of the AMPAR complexes originate from membranes outside postsynaptic density. For example, CPT1C is reported localized in the endoplasmic reticulum of neurons (Sierra et al. 2008) and probably interacts with the AMPAR therein. In addition, many of the identified proteins in Table 2 show strong brain region-specific expression patterns. For example, TARPγ2 is expressed all over the brain, whereas TARPγ8 is strongly expressed in hippocampus and to a lesser extent in cortex but at a very low level in cerebellum. Obviously, this allows for region-specific AMPAR complex formation (Shi et al. 2009), which might be relevant in a functional context. Also, the specific AMPAR complexes might be determined by neuronal activity. Synaptic activity-dependent composition of the AMPAR interactome may directly translate to different synapse efficacies. Future studies should address the spatio-temporal patterns of AMPAR complexes, their activity-dependent composition and functional features and their specific role in synaptic transmission.

Conflict of interest The authors declare that they have no conflict of interest.

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