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Molecular anatomy of the postsynaptic density

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The postsynaptic density (PSD) is a structure composed of both membranous and cytoplasmic proteins localized at the postsynaptic plasma membrane of excitatory synapses. Biochemical and molecular biological studies have identified a number of proteins present in the PSD. Glutamate receptors are important constituents of the PSD and membrane proteins involved in synaptic signal transduction and cell adhesion are also essential components. Scaffolding proteins containing multiple protein interaction motifs are thought to provide the framework of the PSD through their interactions with both membrane proteins and the cytoplasmic proteins. Among the cytoplasmic signaling molecules, calcium-calmodulin-dependent protein kinase II stands out as a major component of the PSD and its dynamic translocation to the PSD in response to neuronal activity is crucial in synaptic signal transduction. Recent advancements in molecular biological, structural and electrophysiological techniques have enabled us to directly measure the number, distribution and interactions of PSD molecules with high sensitivity and precision. In this review, I describe the structure and molecular composition of the PSD as well as the molecular interactions between the major constituents. This information will be combined with recent quantitative analyses of the PSD protein contents per synapse, in order to provide a current view of the PSD molecular architecture and its dynamics.

Keywords: Postsynaptic density; Glutamate receptors; Scaffolding protein; Calcium-calmodulin-dependent protein kinase II; GFP

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Structure of the PSD

Early electron microscopic studies described the ultrastructure of synapses in the central nervous systems (CNS) (Palade and Palay, 1954). The interneuronal chemical synapse consists of a presynaptic element and an apposed postsynaptic element with an intervening synaptic cleft width of 10–20 nm. Both the presynaptic and the postsynaptic membranes display densities on the cytoplasmic faces (Palay, 1958). The presynaptic part of the membrane specialization is referred to as the active zone, as this structure contains the molecules involved in exocytosis of synaptic vesicles. Gray proposed to classify synapses on the basis of their junctional structures (Gray, 1959). He referred to the synapses with prominent postsynaptic membrane thickening as type 1 synapses and those showing less prominent postsynaptic thickening as type

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2 synapses. It turned out that type 1 synapses are the structural characteristics of glutamatergic synapses and type 2 synapses GABAergic and glycinergic synapses (Peters et al., 1991). Thus, the typical postsynaptic density (PSD) is a component of glutamatergic type 1 synapses in the CNS. The majority of glutamatergic synapses are formed onto dendritic spines in the principal neurons of the neocortex (pyramidal neurons) (Spacek and Hartmann, 1983), hippocampus (pyramidal neurons) (Harris and Stevens, 1989) and cerebellum (Purkinje neurons) (Harris and Stevens, 1988). In these neuron types, PSDs and spines are postulated to organize a functional unit, as a large number of experimental evidence indicates that both structures can regulate signal processing in the postsynaptic cytoplasm and there is a correlation in the size of the two structures (Harris and Stevens, 1989). In turn, a majority of interneurons in the neocortex and hippocampus, together with motoneurons in the spinal cord, form excitatory glutamatergic synapses on the dendritic shafts. The basic structures of the PSDs in these neuron types are indistinguishable from neurons possessing dendritic spines.

Using three-dimensional reconstruction of serially sectioned electron microscopic samples, detailed analyses of PSD ultrastructure have been performed. The typical PSD has a disk-like structure with a diameter of 200–500 nm and a thickness of 30–60 nm (Harris et al., 1992; Spacek and Harris, 1998). Holes or perforations are frequently present in larger PSDs, with an associated reduction in granular materials on the cytoplasmic face of the apposed presynaptic membranes. The cytoplasmic boundary of PSDs is relatively diffuse and filamentous structures extending into the interior of the cell can be identified (Gulley and Reese, 1981). In quick-frozen electron microscopic preparations of in vivo synapses, thin filaments running beneath the PSD have been identified and this structure is postulated to be F-actin (Hirokawa, 1989). A submembrane cytoskeletal system is present in spines and is likely to interact with the PSD.

There is wide variability in the PSD area within the same group of synapses. In the case of spine synapses in the area CA1 of the adult hippocampus, the PSD area ranges from 0.008 to 0.54 μm^2. There is also variability in the distribution of PSD area among different types of synapses. The PSD area ranges from 0.003 to 0.23 μm^2 in granule cell spines of the dentate gyrus and 0.039–0.36 μm^2 in cerebellar Purkinje cell spines (Harris and Stevens, 1989; Sorra and Harris, 2000). These quantitative data indicate that the PSD size is determined by both intrinsic properties of cell types and also the previous history of activity in individual synapses.

Detergent disruption of the synaptic membrane preparation allows one to isolate PSDs. The isolated PSDs viewed in the electron microscope show the morphology of circular disks with an average diameter of 300–400 nm, consistent with their dimensions in vivo (Cohen and Siekevitz, 1978). The study of metal replicas of biochemically isolated and freeze-dried PSDs revealed distinct morphological features of the cleft and cytoplasmic surface (Petersen et al., 2003). A dense layer of small particles was characteristic in the cleft surface. In turn, the cytoplasmic surface had a convoluted appearance with irregular protrusions. These morphological differences should be based on distinct molecular compositions of the two surfaces. This point will be discussed in the later sections.

Mass of the PSD

A simple method of estimating the mass of a single PSD is by multiplying the average volume of the PSD by its density. As the diameter and the thickness of the typical PSD are 400 nm and 60 nm, respectively, the volume of a single PSD can be calculated to be 7.5×10^6 nm^3. Protein concentration in the cytoplasm is estimated to be 100 mg/ml, but the protein density within the PSD should be higher than the usual cytoplasm. Therefore, the lowest estimate of the mass of a single PSD will be 7.5×10^{-16} mg, which corresponds to 450 MDa. More precise estimation of the PSD mass was done by Chen and colleagues (2005). In this analysis, preparations of the purified PSD were quickly frozen, freeze-dried and coated with a layer of rotary-shadowed carbon. The specimen was imaged by using a scanning transmission electron microscope (STEM) together with the particles of tobacco mosaic virus as a reference of the molecular mass. The study revealed the molecular mass of the PSDs to be 1.10 ± 0.36 GDa, with average diameters of 360 nm. This PSD mass is twice as large as the above mentioned rough estimation, but this difference can be explained by the higher density of protein molecules within the PSD structure.

Identification of molecules in the PSD

Establishment of biochemical isolation protocols was essential in studying the molecular composition of the PSD. Separation of proteins in the PSD fraction by polyacrylamide gel electrophoresis and subsequent protein sequencing has revealed major constituents of the PSD (Cotman et al., 1974; Cohen et al., 1977). The molecules identified by this strategy include cytoskeletal proteins (actin, tubulin, fodrin and neurofilament proteins), signaling molecules (calmodulin and calcium-calmodulin-dependent protein kinase II (CaMKII)) (Kennedy et al., 1983), membrane receptors (NR2A and NR2B subunits of the *N*-methyl-D-aspartate (NMDA) receptor) (Moon et al., 1994) and scaffolding proteins (PSD-95) (Cho et al., 1992). Generation of polyclonal antibodies against the purified PSD fraction and subsequent screening of an expression library were also used as alternative approaches and these strategies successfully identified the scaffolding proteins SAP90 (an identical protein to PSD-95) and SAP97 (Kistner et al., 1993; Muller et al., 1995).

Possible binding partners of the identified PSD proteins were also isolated efficiently by using the yeast two-hybrid screening system (Kennedy, 2000). The interaction of the NR2 subunits of the NMDA receptor and PSD-95 was identified by this approach (Kornau et al., 1995). Identification of the quaternary complex of Homer, Shank, GKAP and PSD-95, which are able to link the NMDA receptor complex and group I metabotropic glutamate receptors (mGluRs), was another clear example of successful applications of this approach (Tu et al., 1999). Identification of new binding partners by yeast two-hybrid screening and subsequent confirmation of the postsynaptic localization of the target proteins using immunocytochemistry and biochemical fractionation have become a standard approach in the analysis of molecular compositions of the PSD and the interactions between its constituents.

Recent technical progress in high-sensitivity mass spectrometry in combination with automated data analysis systems has enabled the global analysis of the PSD constituents (Husi et al., 2000). Two recent publications identified 492 or 452 proteins from highly purified PSD preparations of rat brains (Jordan et al., 2004; Yoshimura et al., 2004). These analyses revealed proteins belonging to classes of proteins with their major subcellular localization distinct from the PSD, such as those involved in signaling to the nucleus, RNA trafficking and protein translation. It