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Short communication

Nonspecific Association of a 17 kDa Isoform of the Myelin Basic Protein with the Postsynaptic Density Fraction

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The postsynaptic density (PSD), a large protein complex beneath the postsynaptic membrane, is notorious for its 'stickiness'. In order to understand the molecular composition of the PSD fraction, a 17 kDa protein band was isolated by electroelution from SDS-gels, and its partial amino acid sequence was determined from HPLC-purified tryptic peptides of the protein. Surprisingly, the amino acid sequence was identical to that of the previously reported 17 kDa isoform of the myelin basic protein (MBP), an essential protein in CNS myelin formation. Since the protein band represented ~2% of the total proteins in the 1% n-octyl glucoside-insoluble PSD fraction, these results indicate that a significant amount of the 17 kDa isoform of MBP is tightly associated with the PSD during preparation of the PSD fraction.

Keywords: Electroelution, HPLC, Myelin basic protein, Protein Sequencing, PSD, SDS-PAGE.

Introduction

The postsynaptic density (PSD) is a cytoskeletal specialization that is located beneath the postsynaptic membrane. Accumulating data indicate that the PSD is a dynamic structure that is involved in the formation of synapses and the regulation of synaptic transmission (Siekevitz, 1985; Kennedy, 1997). The major components of the PSD fraction were identified as cytoskeletal proteins such as actin, tubulin, and fodrins (Siekevitz, 1985). Recently, many regulatory proteins were also localized to the PSD (Kennedy, 1997, 1998; Siekevitz, 1985). These include neurotransmitter receptors, various protein kinases and phosphatases, and scaffold proteins. The idea is thus supported that the PSD is actively involved in synaptic regulation. Our knowledge, however, about the molecular composition of the PSD is still very limited.

The PSD is described as a collection of filaments with adhering globular proteins (Landis *et al.*, 1974; Cohen *et al.*, 1977). In order to understand the function of the PSD, it is necessary to identify the proteins localized at the PSD. However, the filamentous features of the PSD, together with the hydrophobicity of the adhering proteins, render the PSD very 'sticky' so that some non-PSD proteins are included in the fraction during its preparation. Therefore, not all proteins in the experimental 'PSD fraction' are *bona fide* members of the PSD *in vivo*. Determination of the amino acid sequence is the most direct method to identify unknown proteins (Choi and Rhee, 1998; Moon *et al.*, 1998; Oh and Lee, 1998; Hwang and Lim, 1999). In this study we determined the partial amino acid sequence of a 17 kDa protein (PSD-17), which constitutes ~2% of the 1% n-octyl glucoside (NOG)-insoluble PSD fraction. The sequence information and molecular size indicated that the protein is the 17 kDa isoform of the myelin basic protein (MBP), a major protein of myelin sheath.

Materials and Methods

Preparation of the One-Triton PSD fraction The One-Triton PSD fraction was prepared from an adult rat's (Sprague-Dawley) forebrains as described in Moon *et al.* (1994) and extracted with 1% *n*-octyl glucoside (NOG) at 4°C for 30 min. The soluble (NOG-S) and insoluble (NOG-P) fractions were separated by centrifugation at 200,000 × g for 30 min at 4°C.

Purification of PSD-17 by SDS-PAGE and electroelution The NOG-P fraction was electrophoresed in standard SDS-gels (10%) (Laemmli, 1970). Proteins were stained with 0.2% (w/v) Coomassie R-250 for 30 min in a buffer containing 0.1% SDS and 10 mM Tris-HCl (pH 8.0). Gels were destained in the same solution without Coomassie dye, placed on a florescent light box, and the PSD-17 band was cut with a razor blade. After gel slices were chopped into small pieces (~4 mm), proteins were

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electroeluted in an Elutrap (Schleicher and Schuell) at 250 V for 5-6 hr in a buffer containing 25 mM N-ethylmorpholine (pH8.5) and 0.01% SDS.

High pressure liquid chromatography (HPLC) and peptide sequencing The eluted PSD-17 was concentrated in a Speed-Vac, electrophoresed in a 10% SDS-gel, and the protein band was visualized by staining briefly with Coomassie dye. Proteins in the gel slices were digested with trypsin and the tryptic peptides were purified on a C_{18} reverse phase HPLC column as previously described (Moon *et al.*, 1999). The amino acid sequences were determined by an automated Edman degradation at the Biomolecule Research Group of the Korea Basic Science Institute, Taejon, Korea.

Result and Discussion

In this study, a 17 kDa protein (PSD-17) in the NOG-P fraction was identified as the 17 kDa isoform of MBP. The PSD-17 was present throughout the brain homogenate (BH), synaptosome (Syn), and One-Triton PSD fractions (Fig. 1). In Coomassie-stained gels, the PSD-17 band represented as much as ~2% of the total NOG-P fraction (data not shown), which constitutes a major protein of the fraction. However, the intensity of the PSD-17 band in the NOG-P fraction was not significantly stronger than those in other subcellular fractions, suggesting that the PSD-17 was not specifically associated with the PSD *in vivo*.

The HPLC profile of the tryptic digest of the PSD-17 was simple. Thirteen major peaks were detected in C_{18} RP-HPLC (Fig. 2). Since the average length of tryptic peptides is ~10 amino acids, about 15 tryptic peptides are expected to be derived from the PSD-17. Therefore, this result indicates that the digestion was almost complete. The tryptic peptide of a major HPLC peak (Fig. 2, asterisk) was unambiguously determined for its amino acid sequence (Table 1). In addition, the presence of arginine (R) at -1 position and lysine (K) at the end of the peptide indicated that the peptide was an authentic product of tryptic digestion. A BLAST search (as of January 10, 2000) revealed that the amino acid sequence is perfectly aligned with MBP (Table 1), an essential protein in the CNS myelin formation. So far, four isoforms of MBP, generated by alternative splicing of pre-mRNA (de Ferra *et al.*, 1985), have been reported; 21.5 kDa, 18.5 kDa, 17 kDa, and 14 kDa isoforms (GenBank accession no. CAA10807, CAA10806, CAA10805, CAA10804, respectively). Since the apparent molecular size of the PSD-17 was 17 kDa on SDS-gels, the PSD-associated MBP is most likely to be the 17 kDa isoform. The presence of the 17 kDa isoform of MBP in the PSD fraction was suggested previously by a mixing experiment. When the PSD was isolated from a mixture of myelin fraction

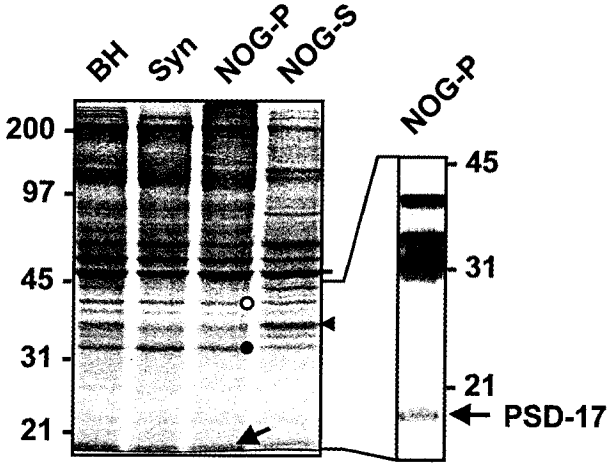

Fig. 1. Electrophoretic profiles of subcellular fractions and the PSD-17 protein band. Brain homogenate (BH), synaptosome (Syn), NOG-insoluble (NOG-P) and -soluble (NOG-S) fractions were prepared from rat forebrains as described under Materials and Methods. Forty micrograms of each of the fractions were electrophoresed in a 10% SDS-gel and stained with Coomassie R-250. Small proteins of the NOG-P fraction were shown at the right panel after a longer run. The positions of PSD-17 are indicated as arrows in the NOG-P lanes. Some of the previously identified proteins, such as actin (bar), glyceraldehyde-3-phosphate dehydrogenase (open circle), adenine nucleotide translocator 1 (closed circle) (manuscript in preparation), and voltage-dependent anion channel 1 (arrowhead), are shown. Molecular sizes were shown in kilodaltons (kDa) at the far left.

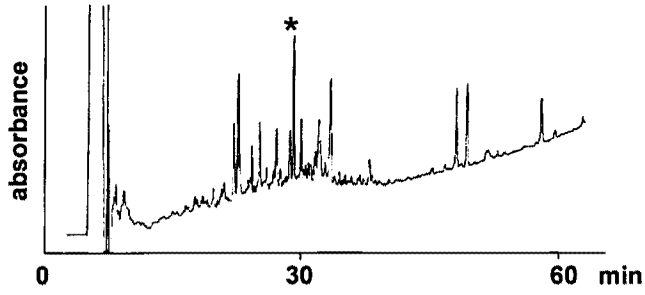

Fig. 2. An HPLC profile of tryptic peptides of the PSD-17. The PSD-17 was purified by electroelution from 10% SDS-gels, digested with trypsin, and the tryptic peptides were chromatographed on a C_{18} RP-HPLC column as described under Materials and Methods. The peptide peak whose amino acid sequence was determined is marked with an asterisk.

Table 1. Amino Acid Sequence.

PSD-17		T	Q	D	E	N	P	V	V	H	F	F	K	
		*	*	*	*	*	*	*	*	*	*	*	*	
MBP	103	R	T	Q	D	E	N	P	V	V	H	F	F	K 115

The amino acid sequence of the peptide peak (asterisk in Fig. 2) was determined by automated Edman degradation at Biomolecule Research Group of the Korea Basic Science Institute, Taejon, Korea. The sequence was aligned with the previously reported 17 kDa isoform of basic myelin protein (GenBank accession no. CAA10805). The arginine (R) at the position corresponding to the -1 position of the peptide is included to show the authenticity of the tryptic peptide.

and synaptosomes, the 17 kDa MBP was co-purified with the PSD and the MBP co-migrated with a 17 kDa protein band in SDS-gels (Cohen *et al.*, 1977). After this observation, Cohen *et al.* (1977) proposed that the 17 kDa protein in the PSD fraction may be the 17 kDa isoform of MBP. However, there has been no direct confirmation of the identity of the PSD-17. This report shows that the PSD-17 is the 17 kDa isoform of MBP.

The PSD is notorious for 'stickiness'. Therefore, one should be cautious in sorting out *bona fide* members of the PSD from contaminants. The PSD fraction used in this work is the One-Triton PSD, which is the pellet fraction after washing the synaptosome-enriched fraction with 0.5% Triton X-100 (Cho *et al.*, 1992). It is known that most proteins that are weakly associated with the PSD 'core' are eliminated by this wash (Cho *et al.*, 1992). In this work, the One-Triton PSD fraction was further washed with another detergent NOG (1%). Even after this wash, about two thirds of the PSD-17 still remained in the pellet fraction (Fig. 1). Although there may be more than one kind of protein in the PSD-17 band, it is likely that most of them are the 17 kDa isoform of MBP, because the major HPLC peak was derived from the MBP. These results indicate, therefore, that a significant amount of the PSD-17 is strongly bound to the PSD 'core'.

Several other non-PSD proteins that were co-purified with the PSD have been reported. Among these, synaptic proteins, such as synapsins and an 150 kDa synaptic vesicle-associated protein, were found in the PSD fraction (Cohen *et al.*, 1977; Walsh and Kuruc, 1992; Moon *et al.*, 1997). However, a presynaptic membrane protein syntaxin and mitochondrial proteins were not present in the PSD fraction (Cohen *et al.*, 1977; Moon *et al.*, 1999). The reasons for the differential contamination of the synaptic proteins in the PSD fraction are unknown. Some presynaptic proteins that are in continuum with the postsynaptic structure may be co-purified with the PSD. Other proteins, including MBP, may bind the PSD nonspecifically in a situation where their original locales are disrupted by homogenation. The present result emphasizes the necessity for immunoelectron and confocal microscopic studies for definite localization of an unknown protein to the PSD *in vivo*.

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