The Role of Zinc in PSD-95 Palmitoyl Modification

Luis Acosta[1], Lili Guerra[2], Marvin Garcia[2], Safiya Syed[3], Ivonn Ruvalcaba[1], Yonghong Zhang[1,2], Xiaoqian Fang[1,3]
[1]UTRGV Biochemistry and Molecular Biology Program, [2]UTRGV College of Science, [3]UTRGV School of Medicine
University of Texas Rio Grande Valley

Introduction

Postsynaptic density-95 (PSD-95) is a membrane-associated guanylate kinase that mediates localization of receptors in the excitatory postsynaptic density. It has been reported that PSD-95 mediates postsynaptic localization of NMDA receptors and anchors postsynaptic AMPAR receptors mainly through its postsynaptic membrane targeting by its N-terminal palmitoylation and serves an essential role in synaptic plasticity. PSD-95 is palmitoylated at two cysteine residues (C3 and C5) at the N-terminus, which is required for its postsynaptic localization. The two histidines (H24, H28) on PSD-95 are critical residues for Zn^{2+}-binding, alongside the two cysteine residues. Recent studies have shown that Ca^{2+}/calmodulin blocks palmitoylation of PSD-95 by binding at the N-terminus of PSD-95, which promotes dissociation of PSD-95 from the postsynaptic membrane and causes loss of surface AMPARs in cultured neurons. Zinc is another metal ion that is found in various areas of the brain and is required for normal development and function. Ionic zinc is highly enriched within the postsynaptic density that diffuses through the Ca^{2+}-permeable channels to influence synaptic plasticity, memory formation and nociception. As an endogenous neuromodulator, zinc plays a role in synaptic transmission and is important in the maintenance of postsynaptic density stability. However, whether or not Zn^{2+} interacts with PSD-95 and regulates PSD-95 modification remain unknown. This study was carried out in human embryonic kidney 293 (HEK-293) cells.

Methods

- Human Embryonic Kidney 293 cells were transfected with PSD-95 and histidine mutant plasmids (H24A, H28A and H24A&H28A). After incubation for 48 hours, the cells were stimulated with 0.1 mM $ZnCl_2$ for 5 min. PBS was used as control in place of $ZnCl_2$.
- Cells were then harvested and the palmitoylation of PSD-95 was assessed using acyl-biotinyl exchange (ABE) method.
- Western blot analysis was conducted to assess the palmitoylation of PSD-95.
- Fluorescein conjugation assay was performed to asses the zinc binding to PSD-95 palmitoylation. Fluorescein dye was added to PSD-95 plasmids in presence and absence of zinc (500 uM). After 1 hour incubation, reactions were then passed over spin desalting columns to remove unconjugated fluorescein dyes.

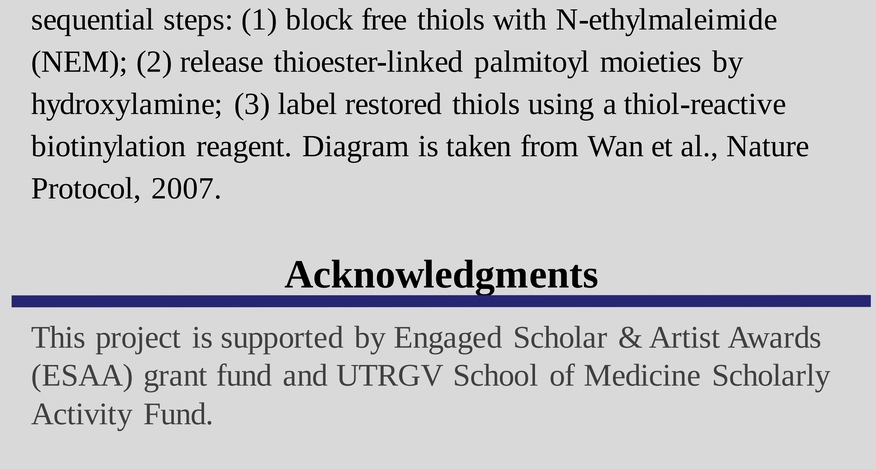

Figure 1. Schematic Diagram of the proteomic acyl-biotinyl exchange (ABE) methodology. ABE method is composed of three sequential steps: (1) block free thiols with N-ethylmaleimide (NEM); (2) release thioester-linked palmitoyl moieties by hydroxylamine; (3) label restored thiols using a thiol-reactive biotinylation reagent. Diagram is taken from Wan et al., Nature Protocol, 2007.

Acknowledgments

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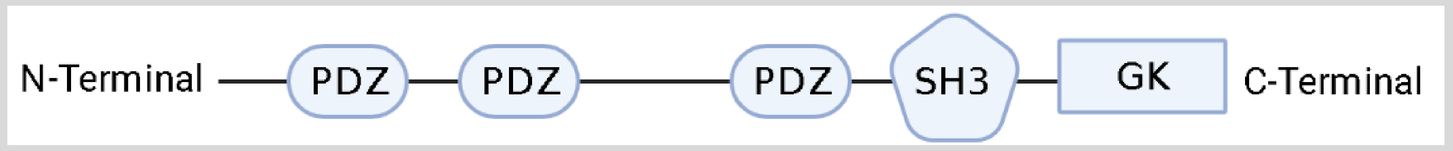

Figure 2. Schematic Structure of PSD-95. The three functional domains of PSD-95 are the three PDZ domains, one SRC homology 3 domain (SH3) and one Guanylate kinase (GK). Diagram was created using BioRender.com

Results

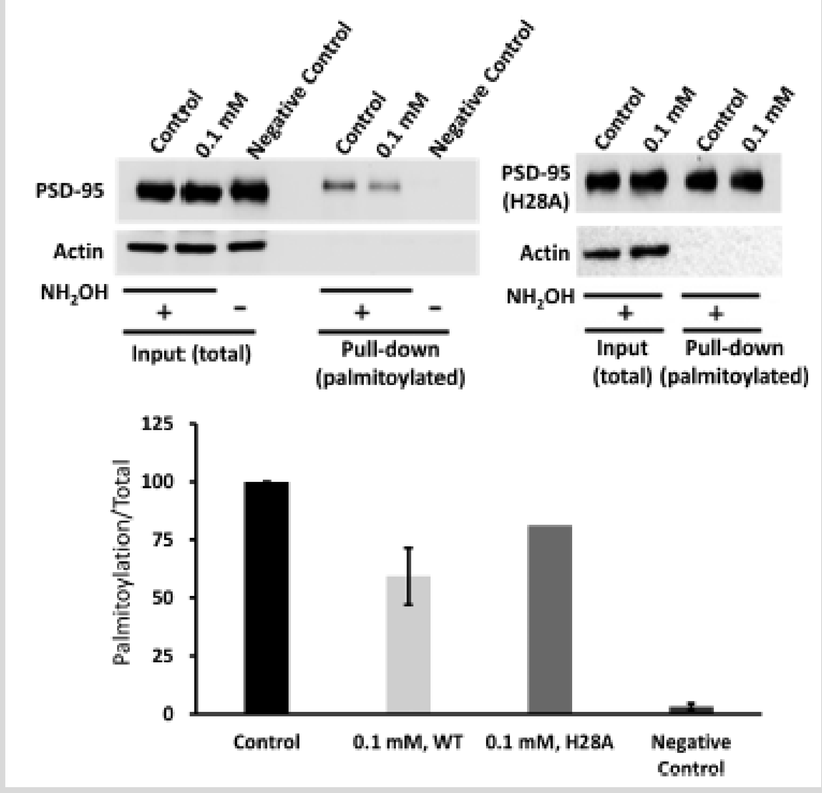

Figure 3. Western Blot analysis of PSD-95 palmitoylation with zinc treatment of wildtype and mutant (H28A and H24A&H28A). HEK293 cells were transfected with PSD-95 and mutant plasmids. After incubation for 48 hours, the cells were stimulated with 0.1 mM $ZnCl_2$ for 5 min and extracted. PBS was used in the control group in place of the $ZnCl_2$. Cells were then harvested and the palmitoylation of PSD-95 was assessed using acyl-biotinyl exchange (ABE) method. Omission of Hydroxylamine (NH_2OH) served as a negative control, preventing the pulldown of PSD-95 exhibiting no palmitoylated PSD-95 signal (right lane). Quantitative analysis was performed using the ratio of palmitoylated/total protein expression. Zinc stimulation decreased palmitoylation level of PSD-95 to about 40% compared to vehicle; however, in histidine mutation it exhibited only to 85%. The PSD-95 double mutation (H24A&H28A) did not have any signal exhibited.

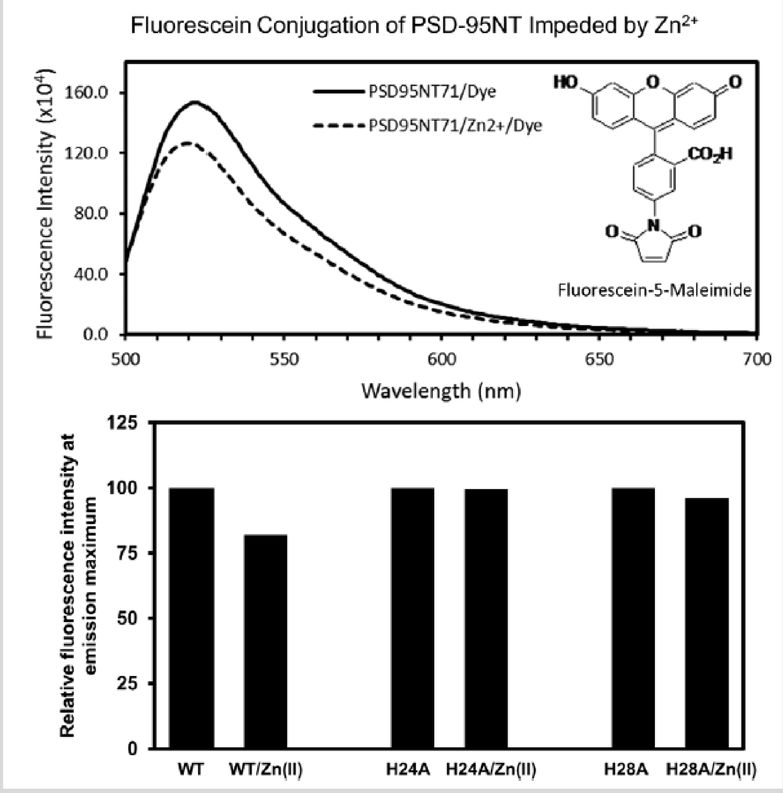

Figure 4. Fluorescein-conjugated modification of PSD-95 is impeded by Zn^{2+} binding. Fluorescence spectrum (upper panel) and relative fluorescence intensity at emission maximum (lower panel) of fluorescein-conjugated PSD-95 and mutants-H24A and H28A in the absence and presence of Zn^{2+}. All samples contain the same concentration of PSD-95 (75 μM). Fluorescence intensity of PSD-95 was decreased ~25% when stimulated with 0.5 mM Zn^{2+} when compared to no treatment. This indicates that zinc decrease the conjugation efficiency of fluorescein dye to PSD-95 due to cysteine blockage. H24A and H28A mutants had a lower binding affinity to zinc than wildtype. This suggests that zinc binding to PSD-95 shifts towards it depalmitoylation and in regard to mutants, zincs inhibitory effect is eliminated due to weakened binding.

Conclusions

Our data showed that zinc stimulation significantly decreased PSD-95 palmitoylation by 40% when compared to control. However, the histidine mutations had less effect on palmitoylation upon stimulation of $ZnCl_2$. The results demonstrated that the Zn^{2+}-induced PSD-95 depalmitoylation was partially abolished in histidine mutants (H24A, H28A, H24A/H28A), which provided additional evidence for this Zn^{2+} regulatory effect on PSD-95.

Future Studies

- The potential effect of the Zn^{2+}-induced depalmitoylation of PSD-95 will be further studied, such as PSD-95 postsynaptic stability and PSD-95 postsynaptic localization.
- The effects of zinc on PSD-95 will be evaluated by comparing the wildtype PSD-95 with the mutant PSD-95 in *in vivo* studies.

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