Neurosci Bull June 1, 2015, 31(3): 331–337. http://www.neurosci.cn
DOI: 10.1007/s12264-014-1519-z

·Original Article·

Zinc binds to and directly inhibits protein phosphatase 2A *in vitro*

Yan Xiong[1,2], Dan-Ju Luo[2], Xiu-Lian Wang[2], Mei Qiu[2], Yang Yang[2], Xiong Yan[2], Jian-Zhi Wang[2], Qi-Fa Ye[1,4], Rong Liu[2,3]

[1]*Wuhan University, Zhongnan Hospital of Wuhan University, Institute of Hepatobiliary Diseases of Wuhan University, Transplant Center of Wuhan University, Hubei Key Laboratory of Medical Technology on Transplantation, Wuhan 430071, China*

[2]*Key Laboratory of Neurological Disease, Ministry of Education, and Department of Pathophysiology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*

[3]*The Institute of Brain Research, Huazhong University of Science and Technology, Wuhan 430030, China*

[4]*The Third Xiangya Hospital of Central South University, Research Center of National Health Ministry on Transplantation Medicine Engineering and Technology, Changsha 410013, China*

Corresponding authors: Rong Liu and Qi-Fa Ye. E-mail: rong.liu@mail.hust.edu.cn, yqf_china@163.com

ABSTRACT

Zinc induces protein phosphatase 2A (PP2A) inactivation and tau hyperphosphorylation through PP2A (tyrosine 307) phosphorylation in cells and the brain, but whether Zn^{2+} has a direct inhibitory effect on PP2A is not clear. Here we explored the effect of Zn^{2+} on PP2A and their direct interaction *in vitro*. The results showed that Zn^{2+} mimicked the inhibitory effect of okadaic acid on protein phosphatase and prevented tau dephosphorylation in N2a cell lysates. PP2A activity assays indicated that a low concentration (10 μmol/L) of Zn^{2+} inhibited PP2A directly. Further Zn^{2+}-IDA-agarose affinity binding assays showed that Zn^{2+} bound to and inhibited $PP2Ac_{(51-270)}$ but not $PP2Ac_{(1-50)}$ or $PP2Ac_{(271-309)}$. Taken together, Zn^{2+} inhibits PP2A directly through binding to $PP2Ac_{(51-270)}$ *in vitro*.

Keywords: zinc; protein phosphatase 2A; direct inhibition

INTRODUCTION

According to clinical surveys, Alzheimer's disease (AD) is the most common type of dementia[1, 2]. It is characterized by the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles within the afflicted brain[3]. The upstream factors and mechanisms leading to tau hyperphosphorylation remain largely unclarified. However, imbalance between the tau kinases involved such as glycogen synthesis kinase 3 and tau phosphatases such as protein phosphatase (PP) 2A has been proposed to play an important role in the abnormal hyperphosphorylation of tau in AD[4-10]. Protein-serine/threonine phosphatase (PP) 2A is found in all eukaryotic cells[11] and plays an important role in the regulation of many cellular events, including viral transformation, translation, replication, the cell cycle, metabolism, cell proliferation, and transcription[12]. PP2A works in heteroassociate form. The PP2A holoenzyme is composed of catalytic C, regulatory/targeting B, and scaffolding/structural A subunits. Currently, the regulation of PP2A activity is not fully elucidated. Holoenzyme composition and post-translational modification have been suggested to modulate PP2A activity. Besides, some intracellular small inhibitory molecules such as I_1^{PP2A} and I_2^{PP2A} may bind to and directly inhibit PP2A[13, 14].

Zinc is a trace metal abundant in the brain. In a previous study, we explored its role in PP2A regulation, and found that it induces PP2A inactivation through Src-dependent PP2Ac (tyrosine 307) phosphorylation

in both cells and rat brain. Meanwhile, the neuronal microtubule-associated protein tau — one of the PP2A dephosphorylating targets — is hyperphosphorylated[15]. This work implies that Zn^{2+} may influence PP2A activity indirectly by changing the tyrosine phosphorylation-related signaling. As a metal ion, Zn^{2+} has been reported to tightly bind to PP2B[26] and directly inhibit the activity of λ Ser/Thr phosphoprotein phosphatase[27]; both phosphatases share sequence identity in the catalytic domain with PP2A[20]. So is it possible that Zn^{2+} also has a direct effect on PP2A? In the present study, we performed *in vitro* studies to answer this question.

MATERIALS AND METHODS

Antibodies and Reagents

Monoclonal antibody against glutathione *S*-transferase (GST) (1:500) was from Tiangen (Beijing, China). Rabbit polyclonal antibodies pS396 (1:1 000) and pS262 (1:1 000) against tau phosphorylated at Ser396 and Ser262, respectively, were from Signalway Antibody (Pearland, TX), pS212 (1:1 000) against tau phosphorylated at Ser212 was from Chemicon Antibody (Massachusetts, MA), and β-actin (1:1 000) was from Epitmics Antibody (Burlingame, CA). Purified PP2A was from Millipore (Billerica, MA). The protein phosphatase assay kit V2460 was from Promega (Fitchburg, WI). Immobilized iminodiacetic acid (IDA) resin was from Pierce (Rockford, IL). Cell culture media were from Gibco (San Diego, CA). $ZnSO_4$ and okadaic acid (OA) were from Sigma (St. Louis, MO).

Expression and Purification of GST-Tagged $PP2Ac_{(1-50)}$, $PP2Ac_{(51-270)}$, and $PP2Ac_{(271-309)}$

The truncated $PP2Ac_{(1-50)}$, $PP2Ac_{(51-270)}$, and $PP2Ac_{(271-309)}$ segments were expressed as thrombin-cleavage GST fusion proteins in *Escherichia coli*. The DNA sequences of $PP2Ac_{(1-50)}$, $PP2Ac_{(51-270)}$, and $PP2Ac_{(271-309)}$ were amplified by PCR with the following primers: sense 1, 5'-cgcggatccccatggacgagaaggtgttcaccaaggagctgg-3' and antisense 1, 5'-aacgcgtcgaccacatcgaacctcttgcacgttggattc-ttttgtc-3'; sense 2, 5'-cgcggatccccccagttactgtctgtggaga-tgtgcatgggcaatttcatgatctcatgg-3' and antisense 2, 5'-aacgc-gtcgaccaccacaacgataacaatagtttggagcactgaaaatcgttac-3'; sense 3, 5'-cgcggatccccaaccaagctgcaatcatggaacttgacgata-ctctaaaatac-3' and antisense 3, 5'-aacgcgtcgacccaggaag-tagtctggggtacgacgagtaacatgtgg-3'. The products were then separately cloned into the pGEX-5X vector. The plasmids were expressed in BL21-CodonPlus-RIL cells (Stratagene, La Jolla, CA), then the cells at OD 0.8 were induced by 0.2 mmol/L isopropyl-β-thiogalactopyranoside for 3 h at 37°C. Pelleted cells were lysed in buffer containing 50 mmol/L Tris-HCl, pH 7.0, 1 mg/L lysozyme, 1.0 mmol/L PMSF, 1:200 protease inhibitor cocktail, 1% TritonX-100, 5 mg/L DNase, and 5 mg/L RNase; and GST-$PP2Ac_{(1-50)}$, GST-$PP2Ac_{(51-270)}$, and GST-$PP2Ac_{(271-309)}$ were solubilized with detergents. The fusion proteins were purified using a B-PER GST fusion protein purification kit (Thermo Scientific, Rockford, IL). Then the purified GST-$PP2Ac_{(1-50)}$, GST-$PP2Ac_{(51-270)}$, and GST-$PP2Ac_{(271-309)}$ and PP2A were incubated with or without 100 μmol/L $ZnSO_4$ for 30 min at 30°C. At the end of incubation, the lysates were collected for western blotting and PP2A activity assays.

Cell Culture and Treatment

Cell culture and incubation of cell lysates with Zn^{2+} were performed as previously described[16]. Mouse neuroblastoma N2a cells were grown to 70%–80% confluence in DMEM/Opti-MEM (1:1) supplemented with 5% fetal bovine serum, 100 units/mL penicillin, and 0.1 mg/mL streptomycin, washed twice, harvested in 0.2 mL of cell lysis buffer (50 mmol/L Tris-HCl, pH 8.0, 2 mmol/L EGTA, 2 mmol/L EDTA, 150 mmol/L NaCl, 1% Triton-X100, 0.5 mmol/L PMSF, 1:1 000 cocktail) and sonicated on ice for 30 min. Then the cell lysates were incubated with or without 100 μmol/L $ZnSO_4$ for 5, 15, or 30 min at 30°C. For analysis of the time- and concentration-dependence of PP2A activity changes caused by Zn^{2+}, N2a cell lysates were incubated with 0, 10, 50, or 100 μmol/L $ZnSO_4$ for 30 min, or with 10 μmol/L $ZnSO_4$ for 0, 10, 30, or 60 min at 30°C. At the end of incubation, the lysates were collected for western blotting and PP2A activity assays.

Western Blotting

The total protein content in cell lysates was measured using a BCA kit (Rockford, IL), and then normalized to 5 μg/μL. The protein samples (30 μg) were resolved on 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were then incubated with primary antibodies, followed by anti-rabbit or anti-mouse IgG conjugated to IRDye™ (800CW; Licor Biosciences,

Lincoln, NE) for 1 h at 25°C, and visualized using the Odyssey Infrared Imaging System (LicorBiosciences).

Protein Phosphatase Activity Assay

The activity of PP2A in cell lysates and purified truncated PP2Ac was assayed using the V2460 Phosphatase Kit (Fitchburg, WI) according to the manufacturer's protocol. Briefly, endogenous phosphate was removed using the spin columns provided, then the N2a cell lysates were normalized for protein content, and 5 μg protein samples in triplicate were incubated with a synthesized phosphopeptide (RRA (pT)VA)[17] for 30 min at 33°C. Then an equal volume of the Molybdate Dye/Additive mixture was added to all wells to stop the reaction. Absorbance at 600 nm or 630 nm was measured with a VersaMax plate reader (Molecular Devices). The PP2A activity was evaluated by the release of phosphate per microgram protein per minute (pmol/μg/min).

Zn^{2+}-IDA-Agarose Affinity Binding Assay

The Zn^{2+}-iminodiacetic acid (IDA)-agarose affinity binding assay was based on previous descriptions[18]. First, IDA column was warmed to room temperature, then 50 μL IDA equilibrated in Buffer A (10 mmol/L Tris-HCl, pH 7.5, 50 mmol/L NaCl, 0.5% TritonX-100) was incubated for 30 min with freshly-prepared 10 mmol/L $ZnSO_4$ at 25°C. The fully Zn^{2+}-charged IDA-agarose was thoroughly washed twice with Buffer A to remove the uncharged Zn^{2+}. Then 50 μL purified GST-$PP2Ac_{(1-50)}$, GST-$PP2Ac_{(51-270)}$, or GST-$PP2Ac_{(271-309)}$ protein was added separately to the pelleted Zn^{2+}-IDA-agarose at room temperature for 30 min. The effluent (designated F) was collected, then the agarose resin was washed 6 times with 50 μL Buffer A, and the wash solution (W1…6) was collected for analysis. Then the bound proteins were eluted from the Zn^{2+}-IDA-agarose 2 or 3 times with 500 μL 100 mmol/L EDTA (E1, E2, or E3). In addition, one group was treated with the same procedure, except for the lack of $ZnSO_4$. All the samples were boiled with sample loading buffer for western blotting using antibody against GST.

Statistical Analysis

Data are presented as mean ± SD and were analyzed with SPSS10.0 software (SPSS Inc., Chicago, IL). Student's *t* test and one-way analysis of variance (ANOVA) followed by the LSD *post hoc* test were used to determine group differences. $P < 0.05$ was considered statistically significant.

RESULTS

Zinc Mimics the Inhibitory Effect of Okadaic Acid on PP2A *in vitro*

To explore whether Zn^{2+} has a direct inhibitory effect on PP2A, we first incubated N2a cell lysates with Zn^{2+} in the absence of exogenous ATP, to exclude the influence of protein kinases on the phosphorylation state of proteins. The results showed that with control incubation, tau was dephosphorylated at Thr212, Ser262, and Ser396 by protein phosphatases, while OA, a potent protein phosphatase inhibitor, prevented the dephosphorylation of tau at 10 nmol/L, a concentration that only inhibits PP2A[19]. Zn^{2+} at 100 μmol/L had an effect similar to OA on tau phosphorylation at all the sites (Fig. 1), suggesting that Zn^{2+} may also directly inhibit PP2A.

Zinc Inhibits PP2A Directly *in vitro*

To further confirm the direct inhibitory effect of Zn^{2+} on PP2A, we incubated N2a cell lysates with Zn^{2+} at 10, 50 or 100 μmol/L without addition of ATP, and assessed the PP2A activity. The results showed that Zn^{2+} inhibited PP2A at all three concentrations, but the higher concentration showed a weaker inhibitory effect (Fig. 2A). We then incubated the lysates with 10 μmol/L Zn^{2+} for 10, 30, or 60 min and found that it time-dependently inhibited PP2A activity (Fig. 2B). These data indicate that Zn^{2+} at a low concentration directly inhibits PP2A.

Zinc-induced PP2A Inactivation Occurs on the $PP2Ac_{(51-270)}$ Segment

Next, we explored the possible mechanism of direct inhibition of PP2A by Zn^{2+}. We found that incubation of full-length PP2A with 100 μmol/L $ZnSO_4$ resulted in significant inhibition of PP2A (Fig. 3B), consistent with the above findings in N2a cell lysates. Further, among the three GST-tagged PP2Ac truncates, $PP2Ac_{(51-270)}$ had the highest phosphatase activity (Fig. 3A), consistent with previous speculation that the catalytic activity of PP2A is located on this segment[20]. In addition, Zn^{2+} significantly inhibited the $PP2Ac_{(51-270)}$ activity (Fig. 3B), with no effect on the other two PP2A truncates, suggesting that the interaction site of Zn^{2+} with PP2A is located on the $PP2Ac_{(51-270)}$ segment.

Zinc Binds Directly to $PP2Ac_{(51-270)}$

The Zn^{2+}-IDA-agarose affinity binding assay showed that

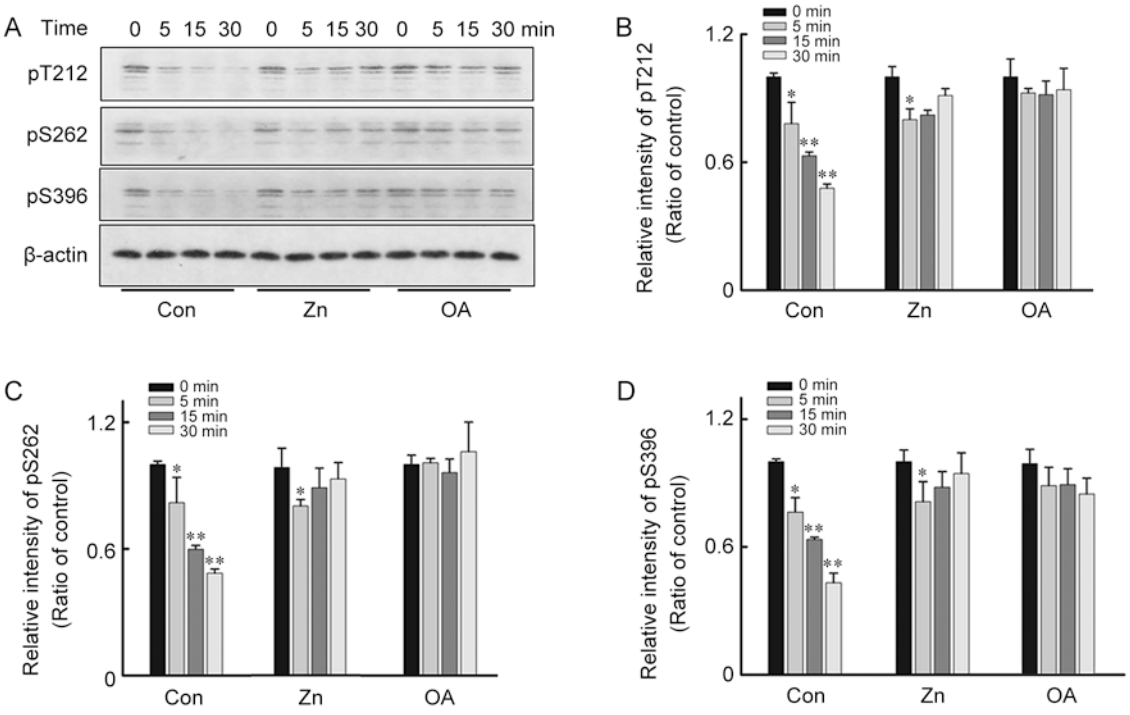

Fig. 1. Zinc mimics the inhibitory effect of okadaic acid (OA) on PP2A *in vitro*. (A) Western blots of the phosphorylation levels of tau at Thr212, Ser262, and Ser396 in N2a cell lysates incubated with or without 100 μmol/L $ZnSO_4$ or 10 nmol/L OA for 0, 5, 15, or 30 min. (B–D) Quantitative analysis normalized against β-actin. All data are expressed as mean ± SD. *P <0.05, **P <0.01 *vs* 0 min group.

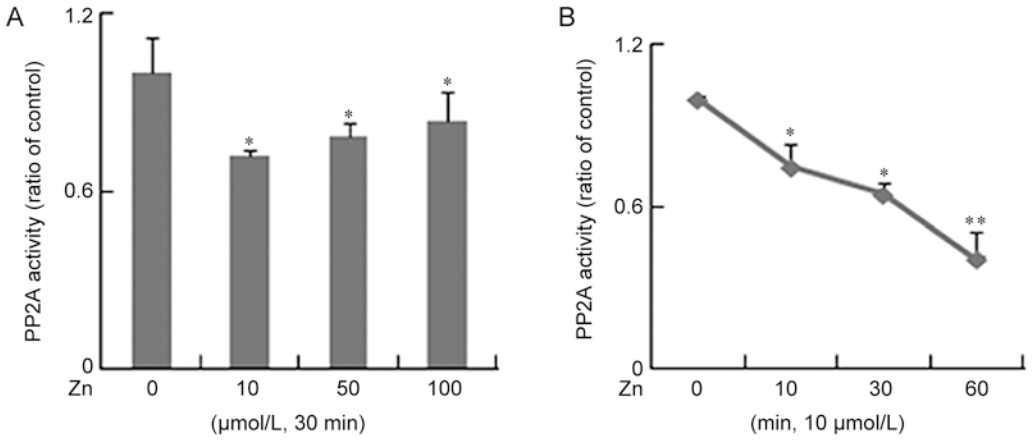

Fig. 2. Zinc inhibits PP2A in a time-dependent manner *in vitro*. (A-B) N2a cell lysates were incubated with 0, 10, 50, or 100 μmol/L $ZnSO_4$ for 30 min (A) or with 10 μmol/L $ZnSO_4$ for 0, 10, 30, or 60 min (B) without ATP; PP2A activity was measured using a protein phosphatase activity assay kit. All data are expressed as mean ± SD. *P <0.05, **P <0.01 *vs* control group.

the recombinant GST-tagged $PP2Ac_{(51-270)}$ bound to Zn^{2+}-charged IDA-agarose (Fig. 4B) but not to uncharged IDA-agarose (Fig. 4A, C). The bound GST-tagged $PP2Ac_{(51-270)}$ protein was eluted by the metal-chelating agent EDTA (100 mmol/L). No Zn^{2+} binding was detected for the other two PP2A segments. These data indicate that Zn^{2+} binds to the $PP2Ac_{(51-270)}$ segment directly and then inhibits PP2A.

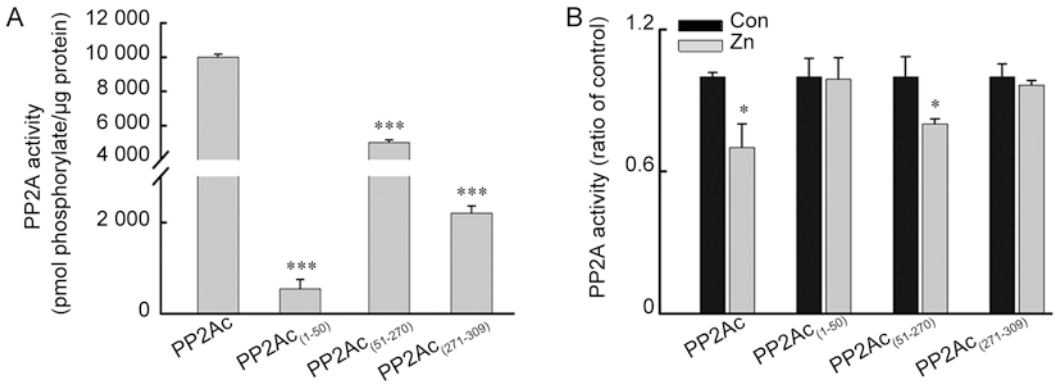

Fig. 3. Recombinant truncated PP2Ac$_{(51-270)}$ possesses biological activity and is inhibited by zinc. (A) Activity of full-length PP2A and purified truncated PP2Ac$_{1-50,\ 51-270,\ 271-309}$ measured by protein phosphatase activity assay. ******P* <0.001 *vs* PP2Ac. (B) Full-length PP2A and purified recombinant PP2Ac$_{1-50,\ 51-270,\ 271-309}$ were separately incubated with 100 μmol/L $ZnSO_4$ for 30 min, and the activity was measured by protein phosphatase activity assay. **P* <0.05 *vs* untreated control.

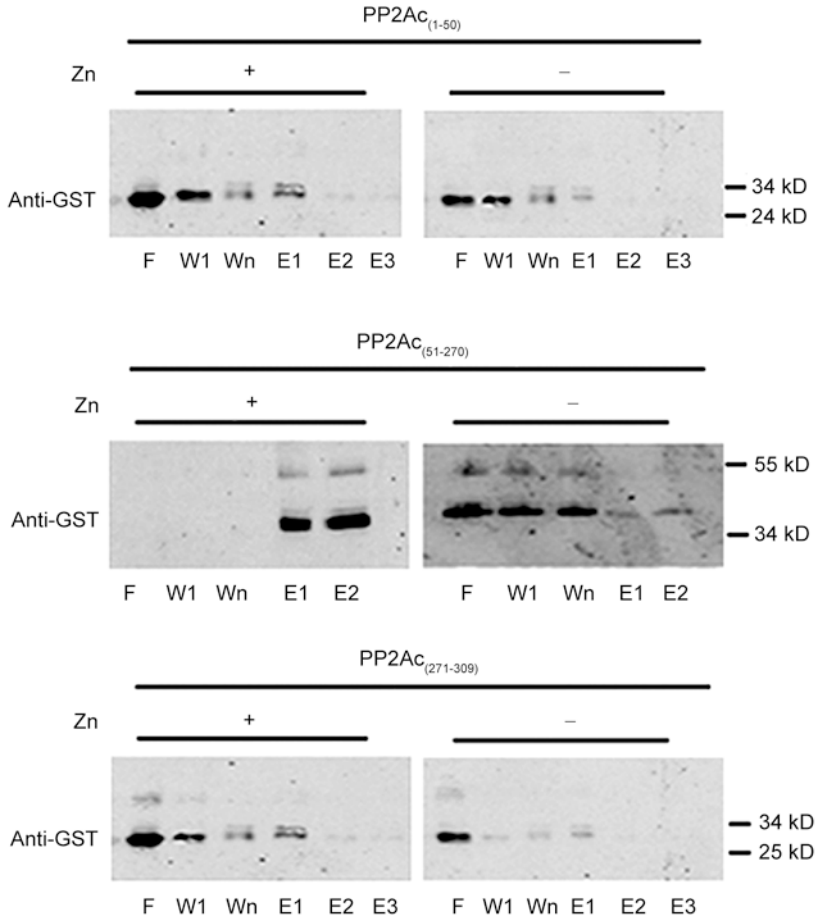

Fig. 4. Zinc binds directly to PP2Ac$_{(51-270)}$. Recombinant truncated GST-PP2Ac$_{1-50,\ 51-270,\ 271-309}$ segments were loaded into IDA-agarose charged with (+) or without (-) Zn^{2+}, then all the fragments were collected separately and underwent Western blotting for GST. Only the elutes of GST-PP2Ac$_{51-270}$ segment loaded into Zn^{2+}-charged DA-agarose show anti-GST bands. F, the effluent; W1 and Wn, wash solutions; E1, E2, and E3, the 1st, 2nd, and 3rd elutes of bound proteins.

DISCUSSION

PP2A is one of the most important serine/threonine protein phosphatases, and is ubiquitous in eukaryotic cells. It plays prominent roles in regulating the cell cycle, signal transduction pathways, morphology, and development[21]. Downregulation of PP2A has been reported in the AD brain[4,5,7], while PP2A suppression is necessary for immortalized cells to complete transformation in carcinogenesis[22]. The PP2A holoenzyme is composed of scaffolding/structural A, regulatory/targeting B, and catalytic C subunits; different B subunits influence its activity by determining the substrate specificity[23]. The activity of the catalytic subunit is affected by many factors. *In vivo,* it is regulated by PP2A inhibitory proteins such as I_1^{PP2A} and I_2^{PP2A}[13, 14]. *In vitro*, the PP2A activity is dramatically inhibited after tyrosine 307 phosphorylation[24, 25]. Xiong *et al.* reported that Zn^{2+} inhibits PP2A activity and induces tauopathy through activating Src-dependent PP2A (tyrosine 307) phosphorylation[15], indicating that Zn^{2+} may inhibit PP2A indirectly through a tyrosine phosphorylation-related signaling pathway.

In this study, we investigated the direct effect of Zn^{2+} on PP2A *in vitro*. We first investigated a possible direct inhibitory effect by comparing the dephosphorylation level of tau in Zn^{2+}- and OA-incubated N2a cell lysates. Tau is a favored substrate of PP2A[10, 28]. *In vitro*, tau is gradually dephosphorylated by many serine/threonine protein phosphatases, of which PP2A contributes the major part[7]. OA is a potent inhibitor of protein phosphatase, and only inhibits PP2A when used at 10 nmol/L *in vitro*[19]. In our study, OA prevented the dephosphorylation of tau at Thr212, Ser262, and Ser396 *in vitro*; all are favored dephosphorylation sites of PP2A, especially Thr212 and Ser262. In addition, Zn^{2+} had an effect on tau dephosphorylation similar to OA, suggesting that Zn^{2+} inhibits PP2A *in vitro*. In our experimental system, exogenous ATP was not added to the cell lysates during incubation, so the effect of tau kinases on tau phosphorylation could be excluded.

Then we assessed the PP2A activity in Zn^{2+}-incubated cell lysates without ATP. The results showed that Zn^{2+} inhibited PP2A at concentrations as low as 10 μmol/L, and the inhibitory effect was time-dependent within 1 h. As new phosphorylation (ATP needed, including tyrosine phosphorylation) was blocked, we interpreted the PP2A inhibition as a direct effect of Zn^{2+}. To confirm this hypothesis, we further incubated purified full-length PP2Ac with Zn^{2+} directly, and found that it reduced PP2A activity by ~30%. To further reveal the underlying mechanism, we cut PP2Ac into several segments and then tested their interactions with Zn^{2+} using a Zn^{2+}-IDA-agarose affinity binding assay. The crystal structure of the PP2A catalytic subunit is not known, however, based on the three-dimensional structure of the PP-1 catalytic subunit, which has a catalytic domain highly homologous with PP2A, the $PP2Ac_{(51-270)}$ segment is thought to cover the catalytic active site and metal coordination center[20]. Furthermore, Wozniak *et al.* reported that the motif of amino-acids 50–270 of PP2Ac contains active site with high affinity with divalent cations[29]. So we tested the interaction of Zn^{2+} with $PP2Ac_{(1-50)}$, $PP2Ac_{(51-270)}$, and $PP2Ac_{(271-309)}$ separately. We found that Zn^{2+} bound with the $PP2Ac_{(51-270)}$ segment, which showed high catalytic activity in the PP2A activity assay, and Zn^{2+} directly inhibited its activity. These results indicate that Zn^{2+} inhibits PP2A directly by binding with $PP2Ac_{(51-270)}$.

In conclusion, Zn^{2+} binds to and inhibits PP2A directly *in vitro,* and this effect occurs on $PP2Ac_{(51-270)}$. Further studies are needed to reveal the detailed mechanism of the interaction.

ACKNOWLEDGEMENTS

This work was supported by Natural Science Foundation of Hubei Province, China (2012FFA044, 2013CFB258) and the National Natural Science Foundation of China (81271403, 81471304, 81261120570).

Received date: 2014-07-07; Accepted date: 2014-10-14

REFERENCES

[1] Mount C, Downton C. Alzheimer disease: progress or profit? Nat Med 2006, 12: 780–784.

[2] Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem 1986, 261: 6084–6089.

[3] Wang JZ, Liu F. Microtubule-associated protein tau in development, degeneration and protection of neurons. Prog Neurobiol 2008, 85: 148–175.

[4] Gong CX, Shaikh S, Wang JZ, Zaidi T, Grundke-Iqbal I, Iqbal

K. Phosphatase activity toward abnormally phosphorylated tau:decrease in Alzheimer disease brain. J Neurochem 1995, 65: 732–738.

[5] Gong CX, Singh TJ, Grundke-Iqbal I, Iqbal K. Phosphoprotein phosphatase activities in Alzheimer disease brain. J Neurochem 1993, 61: 921–927.

[6] Ishiguro K, Shiratsuchi A, Sato S, Omori A, Arioka M, Kobayashi S, *et al*. Glycogen synthase kinase 3 beta is identical to tau protein kinase I generating several epitopes of paired helical filaments. FEBS Lett 1993, 325: 167–172.

[7] Liu F, Grundke-Iqbal I, Iqbal K, Gong CX. Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. Eur J Neurosci 2005, 22: 1942–1950.

[8] Lovestone S, Reynolds CH, Latimer D, Davis DR, Anderton BH, Gallo JM, *et al*. Alzheimer's disease-like phosphorylation of the microtubuleassociated protein tau by glycogen synthase kinase-3 in transfected mammalian cells. Curr Biol 1994, 4: 1077–1086.

[9] Pei JJ, Braak E, Braak H, Grundke-Iqbal I, Iqbal K, Winblad B, *et al*. Distribution of active glycogen synthase kinase3beta (GSK-3beta) in brains staged for Alzheimer disease neurofibrillary changes. J Neuropathol Exp Neurol 1999, 58: 1010–1019.

[10] Wang JZ, Gong CX, Zaidi T, Grundke-Iqbal I, Iqbal K. Dephosphorylation of Alzheimer paired helical filaments by protein phosphatase 2A and 2B. J Biol Chem 1995, 270: 4854–4860.

[11] Cohen P. The structure and regulation of protein phosphatases. Annu Rev Biochem 1989, 58: 453–508.

[12] Mumby MC, Walter G. Protein serine/threonine phosphatases: structure, regulation, and functions in cell growth. Physiol Rev 1993, 73: 673–699.

[13] Li M, Makkinje A, Damuni Z. The myeloid leukemia-associated protein SET is a potent inhibitor of protein phosphatase 2A. J Biol Chem 1996, 271: 11059–11062.

[14] Li M, Guo H, Damuni Z. Purification and characterization of two potent heat-stable protein inhibitors of protein phosphatase 2A from bovine kidney. Biochemistry-Us 1995, 34: 1988–1996.

[15] Xiong Y, Jing XP, Zhou XW, Wang XL, Yang Y, Su XY, *et al*. Zinc induces protein phosphatase 2A inactivation and tau hyperphosphorylation through Src dependent PP2A (tyrosine 307) phosphorylation. Neurobiol Aging 2013, 34: 745–756.

[16] Haase H, Maret W. Intracellular zinc fluctuations modulate protein tyrosine phosphatase activity in insulin/insulin-like growth factor-1 signaling. Exp Cell Res 2003, 291: 289–298.

[17] Donella Deana A, Mac Gowan CH, Cohen P, Marchiori F, Meyer HE, Pinna LA . An investigation of the substrate specificity of protein phosphatase 2C using synthetic peptide substrates; comparison with protein phosphatase 2A. Biochim. Biophys Acta 1990, 1051: 199–202.

[18] Yuan H, Vance KM, Junge CE, Geballe MT, Snyder JP, Hepler JR, *et al*. The serine protease plasmin cleaves the amino-terminal domain of the NR2A subunit to relieve zinc inhibition of the N-methyl-D-aspartate receptors. J Biol Chem 2009, 284: 12862–12873.

[19] Fernandez JJ, Candenas ML, Souto ML, Trujillo MM, Norte M. Okadaic acid, useful tool for studying cellular processes. Curr Med Chem 2002, 9: 229–262.

[20] Goldberg J, Huang HB, Kwon YG, Greengard P, Nairn AC, Kuriyan J. Three-dimensional structure of the catalytic subunit of protein serine/threonine phosphatase-1. Nature 1995, 376: 745–753.

[21] Janssens V, Goris J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. Biochem J 2001, 353: 417–439.

[22] Mumby M. PP2A: unveiling a reluctant tumor suppressor. Cell 2007,130: 21–24.

[23] Goldberg Y. Protein phosphatase 2A: who shall regulate the regulator? Biochem Pharmacol 1999, 57: 321–328.

[24] Chen J, Martin BL, Brautigan DL. Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. Science 1992, 28: 1261–1264.

[25] Liu R, Zhou XW, Tanila H, Bjorkdahl C, Wang JZ, Guan ZZ, *et al*. Phosphorylated PP2A (tyrosine 307) is associated with Alzheimer neurofibrillary pathology. J Cell Mol Med 2008, 12: 241–257.

[26] King MM, Huang CY. The calmodulin-dependent activation and deactivation of the phosphoprotein phosphatase, calcineurin, and the effect of nucleotides, pyrophosphate, and divalent metal ions. Identification of calcineurin as a Zn and Fe metalloenzyme. J Biol Chem 1984, 259: 8847–8856.

[27] Zhuo S, Dixon JE. Effects of sulfhydryl regents on the activity of lambda Ser/Thr phosphoprotein phosphatase and inhibition of the enzyme by zinc ion. Protein Eng 1997, 10: 1445–1452.

[28] Wang JZ, Grundke-Iqbal I, Iqbal K. Restoration of biological activity of Alzheimer abnormally phosphorylated tau by dephosphorylation with protein phosphatase-2A, -2B and -1. Brain Res Mol Brain Res 1996, 38: 200–208.

[29] Wozniak E, Ołdziej S, Ciarkowski J. Molecular modeling of the catalytic domain of serine: threonine phosphatase-1 with the Zn^{2+} and Mn^{2+} di-nuclear ion centers in the active site. Comp Chem 2000, 24: 381–390.