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Effect of Toxic Metal Binding on Tax-Interacting Protein1 (TIP1): A Protein Related to Brain Diseases

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Abstract

Human tax-interacting protein1 (TIP1), also known as glutaminase-interacting protein (GIP), is a small globular protein containing a PDZ domain. PDZ domains are the most common protein-protein interaction modules present in eukaryotes. In humans, TIP1 plays a very important role in many cellular pathways including β-catenin-mediated Wnt signaling, Rho-activator rhotekin-mediated Rho signaling pathway, and glutamate signaling pathway for the normal activity of the central nervous system. TIP1 also regulates potassium channel expression in the plasma membrane and is a binding partner to many proteins including viral oncoproteins, HTLV-1 Tax and HPV16 E6. Since TIP1 is at a pivotal point in many cellular processes through its interaction with a growing list of partner proteins, any impact on the proper functioning of this protein can have severe consequences on the well-being of a living system. Although metals are essential for plants and animals in trace amounts, elevated levels of heavy metals such as arsenic, cadmium, zinc, and lead are toxic causing various health problems including cardiovascular disorders, neuronal damage, renal injuries, and cancer. Here, we report the effect of heavy metals, arsenic and cadmium, on TIP1 conformation using circular dichroism and fluorescence spectroscopy techniques. Our study revealed these metals have a significant impact on the structure of TIP1 even at very low levels.

Keywords

circular dichroism, fluorescence, tax-interacting protein1

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Cellular signaling, a process in which a cell responds to internal/external stimuli, is mediated via protein-protein interactions (PPIs). PPIs are directed through various modules such as SH2, SH3, PDZ, and PH domains present in a protein. PDZ domain,[1,2] named after the proteins, *p*ostsynaptic density −95, *d*iscs large, and *z*ona occludens-1, is a very common PPI in several hundred human proteins.[3] PDZ domain provides a platform where signaling molecules are linked together into large supramolecular signaling complexes.[4] The most important function of PDZ domains involves localization and clustering of ion channels,[5] downstream effectors at the epithelial cell junctions,[6] protein G-coupled receptors,[7] and postsynaptic densities of neurons.[8] In addition, these domains regulate cell polarity and cell-cell communications.[9] Tax-interacting protein1 (TIP1) is a small globular protein containing a single PDZ domain. TIP1 was originally discovered in the human brain while looking for interactors of glutaminase.[10] It is reported to interact with the C-terminus of a growing list of partner proteins[11] including HTLV-1 Tax,[12] HPV E6,[13] Rhotekin,[14] Kir2.3,[15] Glutaminase L,[10] FAS,[16] and β-catenin.[17] Since TIP1 plays pivotal roles in many cellular processes through its interaction with a growing list of partner proteins, any compromise in its structure would affect its function and the well-being of a living cell.

Although metals are essential for plants and animals in trace amounts, elevated levels of metals are toxic to organisms causing various problems including neurocognitive disorders, memory dysfunction, cardiovascular disorders, renal injuries, movement disorders, and cancer.[18,19] Studies have shown that exposure to arsenic during early life can cause loss of brain weight and reduction of neurons and glia.[18]

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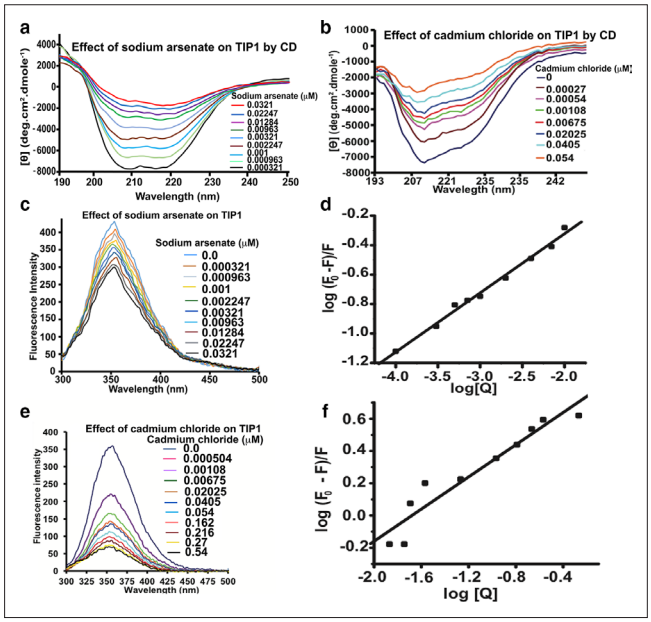

Figure 1. Study of interactions of tax-interacting protein1 (TIP1) with arsenic and cadmium metals by far-UV circular dichroism (CD) and fluorescence spectroscopic techniques. Titration studies of TIP1 with the increasing concentrations of sodium arsenate and cadmium chloride: (a) and (b) represent CD spectra, and (c) and (e) show fluorescence spectra, respectively. Double-log plots of quenching of TIP1 fluorescence by sodium arsenate and cadmium chloride are shown in (d) and (f), respectively.

Cadmium, a heavy transition metal, is a carcinogen as well as a neurotoxin. It enters peripheral and central nervous systems from the olfactory bulb and nasal mucosa leading to neurodegenerative diseases.[20,21] Both of these metals are neurotoxic[22] and affect glutamate signaling, leading to a variety of brain disorders including the above-mentioned diseases.[23]

Since TIP1 plays a vital role in numerous signaling pathways including glutamate, Wnt, Rho, and potassium channel Kir,[11,16,24-26] it is of great interest to understand the effect of metals on TIP1 structure, which in turn can interfere with its function. Here, we report the effect of arsenic and cadmium on the structure of TIP1 using circular dichroism (CD) and fluorescence spectroscopy techniques. Our study revealed that these metals had a significant impact on TIP1 conformation at low levels.

Recombinant TIP1 was expressed in BL21DE3pLys *Escherichia coli*-competent cells using pET-3c vector. TIP1 was purified by size exclusion chromatography in a single step. The effect of individual metal on the secondary structure of TIP1 was monitored by CD spectroscopy. TIP1 was titrated against different concentrations of As^{5+} and Cd^{2+} metal ions. During titration with increasing concentration of metal ions, little precipitation was observed. The CD titration data of metal ions with TIP1 shows that both the metals interact with TIP1 and affect its secondary structure even at very low concentration. With the increasing concentrations of each metal, the helical content of TIP1 was systematically decreased (Figure 1(a) and (b)).

Fluorescence emission spectra of the protein were collected for various concentrations of each metal ion. Tryptophan residue was excited at 280 nm and the emission spectra were recorded in the range of 300 to 500 nm at various concentrations of metal ions during titration with 1 μM TIP1. The fluorescence intensity decreased consistently with each addition of either arsenic or cadmium compound to TIP1. Data clearly showed that quenching did not attain saturation even at higher concentrations of metal ions. For static quenching, equation[27-29] $\log(F_0\text{-}F)/F = \log K + n \log [Q]$ describes the relationship between the fluorescence intensity and the concentration of quenchers, where K is the binding constant, $[Q]$ is the ligand concentration, and n is the number of binding sites per TIP1. The TIP1 and arsenate titration data provided a dissociation constant of 3.0 μM, and the number of binding sites per protein is $n = 0.40$ (Figure 1(c) and (d)). The TIP1-Cd^{2+} titration data yielded a dissociation constant of 2.51 μM, and the number of binding sites per protein is $n = 0.51$ (Figure 1(e) and (f)). The binding affinity of As^{5+} was found almost similar to that of Cd^{2+} indicating that both of the metal ions have similar binding strength to TIP1.

Metal ions play a vital role in life processes. However, above certain levels, metals are toxic to living beings. Human activities have led to the contamination of various sources of water including ground, drinking, and wastewater. Water contamination with lead in 2016 in Flint, Michigan (USA), is a clear example of the effect of toxic metals on public health.[30-32] There are numerous reports on the effects of these toxic metals on human health, especially during the gestation period of pregnant women.[33-35] Human TIP1 is involved in various signaling pathways controlling key biological processes including regulation of the cerebral concentration of neurotransmitter glutamate, β-catenin-mediated Wnt signaling, Rho-activator Rhotekin, and potassium channel Kir2.3 signaling pathway.[11,16,24-26,36]

Considering the importance of TIP1 for the well-being of a living cell, it is of great importance to investigate the effect of toxic metals on the structure of TIP1 and consequently on its function below levels that are currently allowed. We report here the effects of two metals, arsenic and cadmium, on TIP1 structure/conformation. The metal concentrations used for our studies were within the maximum Environmental Protection Agency limit. Thus, the effect seen on the structure of TIP1 at such low metal concentrations has biological significance. Our study clearly indicates that both arsenic and cadmium have a strong interaction with TIP1. Even at low concentration, these metals affect the structure of TIP1 significantly. Metals generally coordinate to proteins through backbone carbonyl oxygens and/or side chains, particularly

the carboxylate groups of Asp and Glu and the ring nitrogen of histidine. A few other amino acid residue side chains can also form coordination to metal ion: Cys, Ser, Thr, Met, Asn, and Gln.[37,38] However, side chains of Cys, His, Asp, and Glu take part in coordination to the metal ions frequently. Although TIP1 lacks Cys residue, it contains 7 Glu, 7 Asp, and 2 His residues. In the structure of TIP1, a majority of these residues are located in loops. Not only are the residues His 90, Asp 91, and Arg 94 located in helix α2, but they also have proper position and orientation for metal coordination (Figure 2(a) and (b)). Thus, these three residues and a water molecule most likely take part in metal coordination presenting a tetrahedral geometry of coordination. Such coordination pattern is common in protein metal coordination.[39] In TIP1, the Asp91-Arg94 salt bridge is very crucial for the organization and stabilization of the PDZ domain.[40] It is very likely that the metal ion breaks the Asp91-Arg94 salt bridge through coordination at this site, thus affecting the structure of TIP1. CD data clearly show that with increasing concentration of both metal ions, the helical content of TIP1 systematically decreases.

The disruption of the salt bridge in α2 helix of TIP1 may have a role in the decrease in protein helicity. From fluorescence data, it is clear that both metal ions bind to TIP1 with high affinity (with K_d = 3.0 μM for As and 2.51 μM for Cd), thus interfering with other interactions involved in structural stabilization of TIP1.

Based on the biophysical data, it is clear that both arsenic and cadmium metal ions affect the structure of TIP1 even at levels lower than currently allowed. Change in TIP1 structure may affect its function in various important biological processes including regulation of the cerebral concentration of the neurotransmitter glutamate, causing numerous health problems in humans.

Combination of several techniques is necessary to understand the chemistry of metal poisoning. By using CD and fluorescence techniques, we have shown here that arsenic and cadmium metals affect the structure of a very important human brain protein, TIP1.

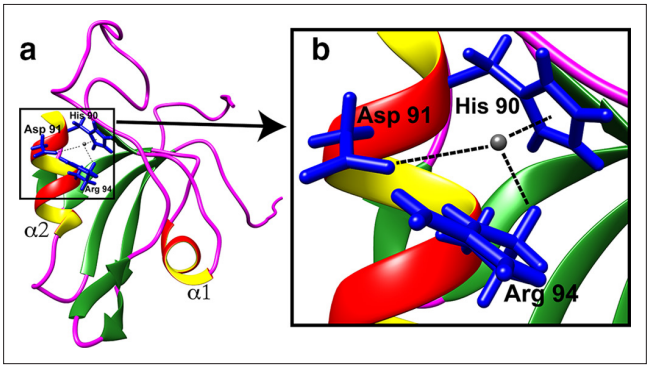

Figure 2. Possible metal-binding site of TIP1. (a) The 3D structure of TIP1 (Protein Data Bank code 2l4s) depicting a metal ion (gray-colored sphere) coordinated to properly oriented residues of α2 helix of TIP1. (b) Enlarged view of proposed metal-binding site of TIP1. The figure was prepared using Chimera.

Experimental

Overexpression and Purification of Recombinant TIP1

Recombinant TIP1/pET-3c plasmid, containing the full-length human TIP1 gene, was transformed into competent *E. coli* BL21DE3plys cells and expressed following previously reported protocol.[16] Briefly, saturated overnight Luria broth (LB)-ampicillin starter culture was diluted (1:40 v/v) in LB media. The diluted fresh culture was grown at 37°C until OD_{600} reached 0.5 to 0.6. Expression was induced with 1 mM isopropyl β-D-1-thiogalactopyranoside and incubated for 4 hours at 30°C with shaking. Cells were harvested by centrifugation. Bacterial cells, in phosphate buffer at pH 8 containing 200 mM NaCl, 4 mM ethylenediaminetetraacetic acid (EDTA), 4% glycerol, and 1 mM phenylmethylsulfonyl fluoride, were lysed using sonication. The lysed cell pellet was separated from the supernatant by centrifugation at 14,000 rpm for 30 minutes. The supernatant containing soluble TIP1 was subjected to purification. Pure TIP1 was obtained in a single step by using size exclusion chromatography with a Sephacryl S-100 column (GE Healthcare, Pittsburgh, PA, USA) fitted to a fast protein liquid chromatography system using 20 mM phosphate buffer containing 150 mM NaCl, 1 Mm EDTA, and 0.1% NaN_3 as the mobile phase.

Fluorescence Spectroscopy

All fluorescence data were collected on Perkin Elmer LS 55 Luminescence spectrofluorometer (Hopkinton, MA, USA) at room temperature at pH 6.5. The emission spectra over the range of 300 to 500 nm with a 1 nm step were recorded by exciting tryptophan fluorescence at 280 nm. Stock solutions of metal ions were prepared in 20 mM phosphate buffer at pH 6.5. To 2 mL of 1 μM protein in 20 mM phosphate buffer at pH 6.5, small aliquots of metal solution were added and fluorescence spectra were recorded at each titration point. The protein concentration was corrected for volume dilution for each titration. The K_d values were calculated following the method.[41] Additionally, the observed emission was corrected for the absorbance of the quencher.

Circular Dichroism Spectroscopy

All CD spectra were recorded on a Jasco J-810 spectropolarimeter (Easton, MD, USA) at room temperature using 0.05 cm quartz cell cuvette. Far-UV CD spectra were collected on 30 μM protein samples in 10 mM phosphate buffer at pH 6.5. The concentration of the protein sample was measured by UV absorption at 280 nm. The far-UV CD data were recorded from 190 to 260 nm at a scan speed of 100

nm/min with 1 second response time. The data were averaged over 50 scans for control and 100 scans for protein sample. The protein concentration was corrected for volume dilution during each titration experiment. The CD ellipticity was converted to mean molar ellipticity per residue following the method described.[42] Titrations of sodium arsenate and cadmium chloride were performed using 51 nM and 27 nM stock solution, respectively.

Structure of TIP1

The structure of TIP1 used in this study was taken from Zoetewey et al.[26]

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Declaration of Conflicting Interests

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References

1. Woods DF, Bryant PJ. ZO-1, DlgA and PSD-95/SAP90: homologous proteins in tight, septate and synaptic cell junctions. *Mech Dev*. 1993;44(2-3):85-89.
2. Ponting CP, Phillips C. DHR domains in syntrophins, neuronal NO synthases and other intracellular proteins. *Trends Biochem Sci*. 1995;20(3):102-103.
3. Luck K, Charbonnier S, Travé G. The emerging contribution of sequence context to the specificity of protein interactions mediated by PDZ domains. *FEBS Lett*. 2012;586(17):2648-2661.
4. Harris BZ, Lim WA. Mechanism and role of PDZ domains in signaling complex assembly. *J Cell Sci*. 2001;114(Pt 18):3219-3231.
5. Le Maout S, Welling PA, Brejon M, Olsen O, Merot J. Basolateral membrane expression of a K^+ channel, Kir 2.3, is directed by a cytoplasmic COOH-terminal domain. *Proc Natl Acad Sci U S A*. 2001;98(18):10475-10480.
6. Wu M, Herman MA. Asymmetric localizations of LIN-17/Fz and MIG-5/Dsh are involved in the asymmetric B cell division in *C. elegans*. *Dev Biol*. 2007;303(2):650-662.
7. Zhong H, Neubig RR. Regulator of G protein signaling proteins: novel multifunctional drug targets. *J Pharmacol Exp Ther*. 2001;297(3):837-845.
8. Fuh G, Pisabarro MT, Li Y, Quan C, Lasky LA, Sidhu SS. Analysis of PDZ domain-ligand interactions using carboxyl-terminal phage display. *J Biol Chem*. 2000;275(28):21486-21491.
9. Zeitler J, Hsu CP, Dionne H, Bilder D. Domains controlling cell polarity and proliferation in the *Drosophila* tumor suppressor Scribble. *J Cell Biol*. 2004;167(6):1137-1146.
10. Olalla L, Aledo JC, Bannenberg G, Márquez J. The C-terminus of human glutaminase L mediates association with PDZ domain-containing proteins. *FEBS Lett*. 2001;488(3):116-122.
11. Banerjee M, Zoetewey DL, Ovee M, et al. Specificity and promiscuity in human glutaminase interacting protein recognition: insight from the binding of the internal and C-terminal motif. *Biochemistry*. 2012;51(35):6950-6960.
12. Rousset R, Fabre S, Desbois C, Bantignies F, Jalinot P. The C-terminus of the HTLV-1 Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. *Oncogene*. 1998;16(5):643-654.
13. Penkert RR, DiVittorio HM, Prehoda KE. Internal recognition through PDZ domain plasticity in the Par-6-Pals1 complex. *Nat Struct Mol Biol*. 2004;11(11):1122-1127.
14. Reynaud C, Fabre S, Jalinot P. The PDZ protein TIP-1 interacts with the Rho effector rhotekin and is involved in Rho signaling to the serum response element. *J Biol Chem*. 2000;275(43):33962-33968.
15. Alewine C, Olsen O, Wade JB, Welling PA. TIP-1 has PDZ scaffold antagonist activity. *Mol Biol Cell*. 2006;17(10):4200-4211.
16. Banerjee M, Huang C, Marquez J, Mohanty S. Probing the structure and function of human glutaminase-interacting protein: a possible target for drug design. *Biochemistry*. 2008;47(35):9208-9219.
17. Kanamori M, Sandy P, Marzinotto S, et al. The PDZ protein tax-interacting protein-1 inhibits beta-catenin transcriptional activity and growth of colorectal cancer cells. *J Biol Chem*. 2003;278(40):38758-38764.
18. Tolins M, Ruchirawat M, Landrigan P. The developmental neurotoxicity of arsenic: cognitive and behavioral consequences of early life exposure. *Ann Glob Health*. 2014;80(4):303-314.
19. Rehman K, Fatima F, Waheed I, Akash MSH. Prevalence of exposure of heavy metals and their impact on health consequences. *J Cell Biochem*. 2018;119(1):157-184.
20. Jiang L-F, Yao T-M, Zhu Z-L, Wang C, Ji L-N. Impacts of Cd(II) on the conformation and self-aggregation of Alzheimer's tau fragment corresponding to the third repeat of microtubule-binding domain. *Biochim Biophys Acta*. 2007;1774(11):1414-1421.
21. Okuda B, Iwamoto Y, Tachibana H, Sugita M. Parkinsonism after acute cadmium poisoning. *Clin Neurol Neurosurg*. 1997;99(4):263-265.
22. Chen P, Miah MR, Aschner M. Metals and neurodegeneration. *F1000Res*. 2016;5:F1000.
23. Tyler CR, Allan AM. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. *Curr Environ Health Rep*. 2014;1(2):132-147.
24. Zencir S, Banerjee M, Dobson MJ, et al. New partner proteins containing novel internal recognition motif for human glutaminase interacting protein (hGIP). *Biochem Biophys Res Commun*. 2013;432(1):10-15.
25. Zencir S, Ovee M, Dobson MJ, Banerjee M, Topcu Z, Mohanty S. Identification of brain-specific angiogenesis inhibitor 2 as an interaction partner of glutaminase interacting protein. *Biochem Biophys Res Commun*. 2011;411(4):792-797.

26. Zoetewey DL, Ovee M, Banerjee M, Bhaskaran R, Mohanty S. Promiscuous binding at the crossroads of numerous cancer pathways: insight from the binding of glutaminase interacting protein with glutaminase L. *Biochemistry*. 2011;50(17):3528-3539.
27. Feng XZ, Jin RX, Qu Y, HeXW. Study on the ion effect on the binding interaction between HP and BSA. *Chem J Chin Univ*. 1996;17:866–869.
28. LiJC, Li N, Wu QH, et al. Study on the interaction between clozapine and bovine serum albumin. *J Mol Struct*. 2007;833(1-3):184-188.
29. Wei XF, Liu HZ. The interaction between Triton X-100 and bovine serum albumin. *Chin J Anal Chem*. 2000;28(6):699-701.
30. Fortenberry GZ, Reynolds P, Burrer SL, et al. Assessment of Behavioral Health Concerns in the Community Affected by the Flint Water Crisis - Michigan (USA) 2016. *Prehosp Disaster Med*. 2018;33(3):256-265.
31. Muhammad M, De Loney EH, Brooks CL, Assari S, Robinson D, Caldwell CH. "I think that's all a lie…I think It's genocide": applying a critical race praxis to youth perceptions of flint water contamination. *Ethn Dis*. 2018;28(Suppl 1):241-246.
32. Pieper KJ, Tang M, Edwards MA. Flint water crisis caused by interrupted corrosion control: investigating “Ground Zero” home. *Environ. Sci. Technol.*. 2017;51(4):2007-2014.
33. Omeljaniuk WJ, Socha K, Soroczynska J, et al. Cadmium and lead in women who miscarried. *Clin Lab*. 2018;64(1):59-67.
34. Schwalfenberg G, Rodushkin I, Genuis SJ. Heavy metal contamination of prenatal vitamins. *Toxicol Rep*. 2018;5:390-395.
35. Tulic L, Vidakovic S, Tulic I, Curcic M, Bulat Z. Toxic metal and trace element concentrations in blood and outcome of in vitro fertilization in women. *Biol Trace Elem Res*. 2018;186:1-11.
36. Mohanty S, Ovee M, Banerjee M. PDZ domain recognition: insight from human tax-interacting protein 1 (TIP-1) interaction with target proteins. *Biology*. 2015;4(1):88-103.
37. Glusker JP. Structural aspects of metal liganding to functional groups in proteins. *Adv Protein Chem*. 1991;42:1-76.
38. Yamashita MM, Wesson L, Eisenman G, Eisenberg D. Where metal ions bind in proteins. *Proc Natl Acad Sci U S A*. 1990;87(15):5648-5652.
39. Regan L. Protein design: novel metal-binding sites. *Trends Biochem Sci*. 1995;20(7):280-285.
40. Tochio H, Mok YK, Zhang Q, Kan HM, Bredt DS, Zhang M. Formation of nNOS/PSD-95 PDZ dimer requires a preformed beta-finger structure from the nNOS PDZ domain. *J Mol Biol*. 2000;303(3):359-370.
41. Mazumder S, Dahal SR, Chaudhary BP, Mohanty S. Structure and function studies of Asian corn borer *Ostrinia furnacalis* pheromone binding protein2. *Sci Rep*. 2018;8(1):17105.
42. Chaudhary B, Mazumder S, Mohanty S. Production and biophysical characterization of a mini-membrane protein, Ost4V23D: a functionally important mutant of yeast oligosaccharyltransferase subunit Ost4p. *Protein Expr Purif*. 2017;139:43-48.