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Editorial

Modulators, Metals and Toxicity: A Tale of Two Proteins

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Sometimes research in seemingly different areas can bring about unexpected connections. I relate my past experience with research on two very different proteins that share some general properties and connections in the areas of toxicity and cellular regulation.

Second messenger theory was in its infancy when Wai Yiu "George" Cheung (1933-1998) discovered one of the first multi-enzyme modulators [1,2]. Initially referred to as calcium-dependent regulator (CDR) protein, it was later christened by George, as calmodulin since its modulatory function was dependent on binding to calcium ions [3]. A small protein of approximately 16.7 kDa, calmodulin was initially found to activate cyclic nucleotide phosphodiesterase Type I [2]. Follow-up studies by several investigators showed that calmodulin also interacted with, and regulated other proteins including adenylyl cyclase Type I [4], myosin light chain kinase [5], phosphorylase kinase [6], calcineurin (a phosphatase [7]), and Ca^{2+}-ATPase [8]. So as a modulator of multiple targets, calmodulin was seen to regulate several pathways in various cell types affecting many physiological systems [9]. These functions range from contractile processes and cyclic nucleotide metabolism to apoptosis, immune response and both long and short-term memory [10,11].

Structural studies of calmodulin revealed four EF hand motifs allowing for the binding of four calcium ions [12]. Binding of calcium ions leads to an open conformation enabling higher affinity to target enzymes. Since calmodulin is a calcium-binding protein, George hypothesized that it might bind other metal ions as well. It was not lost upon him that binding of certain metals to a multi-enzyme regulator could, in theory, be used to describe a mechanism for metal toxicity. Since the normal modulatory function of calmodulin is dependent on intracellular calcium levels, exposure to high concentrations of other metals might be capable of displacing calcium ions from the protein, thus affecting regulatory function [13]. Initial work in his lab revealed that several metals bind to calmodulin, including terbium, cadmium, lead, and mercury [13,14]. As a postdoc in George's lab at the time, I had involvement in these studies, some of which revealed a correlation between metal toxicity, inhibition of calmodulin function, and metal ion radii [13]. Of particular interest was the finding that metal ions most effective in displacing calcium from calmodulin binding sites were those with ionic radii closest in size to the calcium ion, such as terbium, lanthanum, samarium, and cadmium [13]. Binding of metals other than calcium might also represent an alternative mechanism of control of specific cellular processes by calmodulin [15].

Many proteins bind metal ions to execute their functions; some, like calmodulin, are regulatory proteins. Other proteins have unknown functions, but like calmodulin, interact with multiple targets. Fast-forward sixteen years from Dr. Cheung's lab at St. Jude Children's Hospital in Memphis, Tennessee to an industrial research lab at a major pharmaceutical company in Wilmington, Delaware. As a project leader for a Parkinson's disease initiative, I examined a protein like the one described above. Alpha-synuclein, a small protein of approximately 14.5 kDa is associated with the pathology of Parkinson's disease and related conditions known as synucleinapathies [16]. Comprising as much as one percent of the cytosolic protein in brain tissue, alpha-synuclein lacks a stable three-dimensional structure and forms aggregates in the disease state, that are believed to be toxic to neurons [17]. Indeed, these aggregates are a primary component of Lewy bodies, cellular inclusions found in Parkinson's disease, the second most common neurodegenerative disorder in people older than sixty-five [17,18]. Alpha- synuclein has also been found to bind various metal ions, some of which (Al^{3+}, Cu^{2+}) trigger alpha-synuclein aggregation in solution [19]. Interestingly, an elegant study by Ducic., *et al.* [20] indicates that alpha-synuclein may serve as an intracellular store for manganese ions suggesting a regulatory mechanism for neurotoxicity.

Although the function of alpha-synuclein is unknown, several hypotheses have been proposed based on its structure, protein interactions, and intracellular location. As an example, alpha- synuclein shares a 40% homology with regions of the chaperone-like proteins 14-3-3 [21] and in aggregate form, binds to 14-3-3η [22]. If alpha-synuclein functions as a chaperone, this would complement its proposed role in vesicular trafficking via a reported interaction with VAMP2, a component of the SNARE complex [23]. Localization of alpha-synuclein at pre-synaptic terminals further validates this proposed function [24]. The interaction of alpha-synuclein with the dopamine transporter [25] and with the parkin-binding protein, synphilin I [26], suggest a role in the pathological mechanism for Parkinson's disease.

The intent of this commentary has been to provide a picture of two proteins from different systems that I have had the opportunity to study, and examine their roles in the context of cellular regulatory processes and toxicity. Calmodulin and alpha-synuclein are distinctly different proteins and in this regard the multiple protein-protein interactions involving alpha-synuclein should not be compared with the binding of target proteins to calmodulin. While both are small proteins that bind metal ions, calmodulin's role as a regulator is well-documented. The role of alpha-synuclein in cellular regulation is less clear and binding to metal ions may not correlate with a specific function. The structure of calmodulin follows precise three-dimensional conformations upon binding calcium ions, while alpha-synuclein is natively unfolded in the monomeric form. And while alpha-synuclein has a (low affinity) calcium-binding site [27], it is not clear that it is important in a functional sense. However, some comparisons are instructive. If alpha-synuclein acts as a chaperone, it may also sub serve a regulatory function. Many of the studies cited above support this idea. In Wilmington, we showed that alpha-synuclein-induced toxicity could be inhibited by over-expression of the E3 ligase parkin in cell culture, supporting earlier observations [28,29]. While at Lincoln University (PA), I presented some preliminary data showing that alpha synuclein inhibits auto-ubiquitylation of the E3 ligase, HRD1 [30], a protein potentially implicated in Parkinson's disease [31]. So, is alpha-synuclein a modulator in the same manner as calmodulin? Exposure to heavy metals probably affects calmodulin and alpha-synuclein by different mechanisms, but binding of these metal ions to both proteins induces conformational changes that cause deviations in regulatory events, with specific pathologies as a possible consequence.

And there is more. At least one report provides evidence for an interaction between calmodulin and alpha-synuclein [32]. Photo cross-linking of cytosolic proteins from bovine brain tissue indicate calcium-dependent binding of calmodulin to alpha-synuclein that may regulate dopamine release. A decrease in calcium-binding proteins (including calmodulin) in cell bodies of neurons where alpha-synuclein aggregates occur, may explain the histopathology observed in Parkinson's disease [33]. If alpha synuclein is a modulator, is it regulated by another modulator?

Calmodulin and alpha-synuclein are very different proteins that interact with metal ions and probably with each other. Although the contrasts in terms of their structures are striking, their roles in cellular function may overlap suggesting a connectivity to a common endpoint in terms of regulation and toxicity. For regulation, the proteins could act to control dopamine release [32] or other processes. Toxicity could involve metal-induced aggregation of alpha-synuclein that may cause a decreased expression of calmodulin [33] leading to a loss of function. Alternatively, heavy metal-induced inhibition of calmodulin could block binding to alpha-synuclein, again leading to a loss of function. These scenarios are testable, but will require a better understanding of the physiological function of alpha-synuclein and the relevance, if any, of calmodulin-alpha-synuclein interactions. I think George would have found the potential connections interesting.

Bibliography

1. Cheung WY. "Cyclic 3',5'-nucleotide phosphodiesterase: preparation of a partially inactive enzyme and its subsequent stimulation by snake venom". *Biochim Biophys Acta* 191.2 (1969): 303-315.

2. Cheung WY. "Cyclic 3',5'-nucleotide phosphodiesterase. Evidence for and properties of a protein activator". *The Journal of Biological Chemistry* 246.9 (1971): 2859-2869.

3. Wallace RW and Cheung WY. "Calmodulin". *The Journal of Biological Chemistry* 254 (1979): 6564-6571.

4. Brostrom CO., *et al*. "Identification of a calcium-binding protein of brain adenylate cyclase and cyclic nucleotide phosphodiesterase". *PNAS USA* 72.1 (1975): 64-68.

5. Blumenthal DK and Stull JT. "Activation of skeletal muscle myosin light chain kinase by calcium (2+) and calmodulin". *Biochemistry* 19 (1980): 5608-5614.

6. Picton C., *et al.* "The regulation of muscle phosphorylase kinase by calcium ions, calmodulin and troponin-C". *Cell Calcium* 2.4 (1981): 281-294.

7. Hubbard MJ and Klee CB. "Calmodulin binding by calcineurin. Ligand-induced renaturation of protein immobilized on nitrocellulose". *The Journal of Biological Chemistry* 262.31 (1987): 15062-15070.

8. Luthra MG. "Trifluoperazine inhibition of calmodulin-sensitive Ca2+ -ATPase and insensitive (Na+ +K+)- and Mg2+ -ATPase activities of human and rat red blood cells". *Biochim. Biophys. Acta* 692 (1982): 271-277.

9. Cheung WY. "Calmodulin". *Scientific American* 246 (1982): 62-70.

10. Vetter SW and Leclerc E. "Novel aspects of calmodulin target recognition and activation". *European Journal of Biochemistry* 270.3 (2003): 404-414.

11. McDowall J. "Calmodulin". *InterPro Protein Archive* (2003-01-01).

12. Finn BE and Forsen S. "The evolving model of calmodulin structure, function and activation". *Structure* 3.1 (1995): 7-11.

13. Chao S-H., *et al.* "Activation of calmodulin by various metal cations as a function of ionic radius". *Molecular Pharmaceutics* 26.1 (1984): 75-82.

14. Suzuki Y., *et al.* "Stimulation of calmodulin by cadmium ion". *Arch Toxicol* 57.3 (1985): 205-211.

15. Mills JS and Johnson JD. "Metal ions as allosteric regulators of calmodulin". *The Journal of Biological Chemistry* 260.28 (1985): 15100-15106.

16. Spillantini MG and Goedert M. "The alpha-synucleinopathies: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy". *Annals of the New York Academy of Sciences* 920 (2000): 16-27.

17. Spillantini MG., *et al.* "α-Synuclein in Lewy bodies". *Nature* 388.6645 (1997): 839-840.

18. Brown DR. "Oligomeric alpha-synuclein and its role in neuronal death" *IUBMB Life* 62.5 (2010): 334-339.

19. Carboni E and Lingor P. "Insights on the interaction of alpha-synuclein and metals in the pathophysiology of Parkinson's disease". *Metallomics* 7.3 (2015): 395-404.

20. Ducic T., *et al.* "Alpha-synuclein regulates neuronal levels of manganese and calcium". *ACS Chemical Neuroscience* 6.10 (2015): 1769-1779.

21. Ostrerova N., *et al.* "α-Synuclein Shares Physical and Functional Homology with 14-3-3 Proteins". *The Journal of Neuroscience* 19.14 (1999): 5782-5791.

22. Plotegher N., *et al.* "The chaperone-like protein 14-3-3η interacts with human α-synuclein aggregation intermediates rerouting the amyloidogenic pathway and reducing α-synuclein cellular toxicity". *Human Molecular Genetics.* 23.21 (2014): 5615-5629.

23. Burre J., *et al.* "Alpha-synuclein promotes SNARE-complex assembly *in vivo* and *in vitro*". *Science* 329.5999 (2010): 1663-1667.

24. Murphy DD., *et al.* "Synucleins Are Developmentally Expressed, and α-Synuclein Regulates the Size of the Presynaptic Vesicular Pool in Primary Hippocampal Neurons". *The Journal of Neuroscience* 20.9 (2000) 3214-3220.

25. Sidhu A., *et al.* "Alpha-Synuclein regulation of the dopaminergic transporter: a possible role in the pathogenesis of Parkinson's disease". *FEBS Lett* 565.(1-3) (2004) 1-5.

26. Chung KK., *et al.,* "Parkin ubiquitinates the α-synuclein–interacting protein, synphilin-1: implications for Lewy-body formation in Parkinson disease". *Nature Medicine* 7 (2001): 1144-1150.

27. Nielsen MS., *et al.* "Ca^{2+} Binding to α-Synuclein Regulates Ligand Binding and Oligomerization". *The Journal of Biological Chemistry* 276.25 (2001): 22680-22684.

28. Oluwatosin-Chigbu Y., *et al.* "Parkin suppresses wild-type alpha-synuclein-induced toxicity in SHSY-5Y cells". *Biochemical and Biophysical Research Communications* 309.3 (2003): 679-684.

29. Petrucelli L., *et al.* "Parkin protects against toxicity associated with mutant alpha-synuclein: proteosome function selectively affects catacholaminergic neurons". *Neuron* 36.6 (2002) 1007-1019.

30. Zysk JR. *et al.* "Inhibition of HRD1 ligase activity by alpha synuclein". 44th Annual Meeting of the Society for Neuroscience, Washinton, DC (2014).

31. Omura T., *et al.* "A ubiquitin ligase HRD1 promotes the degradation of Pael receptor, a substrate of Parkin". *Journal of Neurochemistry* 99.6 (2006): 1456-1469.

32. Martinez J., *et al.* "Parkinson's disease-associated α-synuclein is a calmodulin substrate". *The Journal of Biological Chemistry* 278.19 (2003): 17379-17387.

33. Hurley MJ., *et al.* "Parkin*son*'s disease is associated with altered expression of Ca_v1 channels and calcium-binding proteins". *Brain* 136 (2013): 2077-2097.

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