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Novel Cu²⁺ binding site in C₂A Domain of Synaptotagmin I. F. Guo, D. Rajalingam, T.K.S. Kumar, J. Sakon, Univ. of Arkansas, Fayetteville, AR 72701, USA.

Synaptotagmins are synaptic vesicle membrane proteins which consist of calcium binding C₂A and C₂B domains. They are involved in membrane traffic and signal transduction as neurotransmitter release through its calcium dependent interactions with syntaxin and phospholipids. C₂A is an ~18kD all-beta-sheet protein. Structure of C₂A with calcium is well characterized by both NMR and X-rays. Recent studies show that C₂A domain has a high binding affinity for copper and triggers multiprotein complex release like human acidic fibroblast growth factor (hFGF-1). The 1.4 Å resolution structure of C₂A with Cu²⁺ shows only single histidine ligating the metal. Compared to our 1.2 Å resolution structure of C₂A, a conformational change takes place upon Cu²⁺ binding. ¹⁵N-¹H HSQC titration confirmed the Cu²⁺ bound in the vicinity of His254 which is located in the loop between beta-strands 7 and 8. Results from isothermal calorimetry (ITC) show that C₂A has extraordinary binding affinity with copper (in the nanomolar range). The elucidation of copper binding site in C₂A indicates that the synaptotagmin I may switch function through binding different metal ions and it is an effective strategy to inhibit the FGF-1-induced pathogenesis by prevention of the formation of the copper-bound state of C₂A.