

Single Histidine of Synaptotagmin I C2A Domain coordinates with Two Cu²⁺

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In the non-classical secretion route, Cu²⁺ ions are required for the formation of multiprotein release complex which consists of fibroblast growth factor, S100A13, and synaptotagmin I (Syt I). C2A is a Ca²⁺ binding domain of Syt I. Previously NMR and ITC studies indicated that three Cu²⁺ ions bound to Ca²⁺ sites and a single Cu²⁺ ion was bound to a remote site in C2A. Here we identified Cu²⁺ binding sites at 1.4Å resolution by utilizing anomalous signals ($\lambda=1.37624\text{\AA}$). Not one but two Cu²⁺ ions were located at the remote site, and both coordinated with a N~~1~~2 of His254. In site 1 (occ=0.7), His254 is positioned by Asp251, whereas it is positioned by carbonyl group of Val255 in site 2 (occ=0.3). Exposed binding sites could be functionally important as it is hypothesized that Cu²⁺ ions are involved in the organization of the FGF multiprotein release complex. The loop 1 that constitutes the Ca²⁺ binding sites can adopt either an 'open' (unbound) or a 'close' (bound) conformation. C2A in the presence of Cu²⁺ adopts an 'open' conformation. Mn²⁺ on the other hand was able to 'close' the loop to bind to one of the Ca²⁺ sites. Structures were analyzed to explain discrepancies in the number and positions of Cu²⁺ binding sites in vitro and in the crystal.

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