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**Structural Basis of Citrate-dependent and Heparan Sulfate-mediated Cell Surface Retention of Cobra Cardiotoxin A3.** C.-J. Chen <sup>a,c</sup>, S.-C. Lee <sup>b</sup>, H.-H. Guan <sup>a,b</sup>, C.-H. Wang <sup>b</sup>, W.-N. Huang <sup>d</sup>, S.-C. Tjong <sup>b</sup>, W.-g. Wu <sup>b</sup>, <sup>a</sup>Biology Group, National Synchrotron Radiation Research Center, Hsinchu, Taiwan; <sup>b</sup>Dept. of Bioinformatics Structural Biology; <sup>c</sup>Dept. of Physics Structural Biology Program, National TsingHua Univ., Hsinchu, Taiwan.

Anionic citrate is a major component of venom, but the role of venom citrate in toxicity is poorly understood other than its inhibitory effect on the cation-dependent action of venom toxins. By immobilizing CHO cells in microcapillary tubes and heparin on sensor chips, we demonstrated that heparan sulfate (HS)-mediated cell retention of the major cardiotoxin (CTX) from the Taiwan cobra, CTX A3, near membrane surfaces is citrate dependent. The CTX A3-heparin hexasaccharide complex structure at 2.4 Å resolution revealed a molecular mechanism for toxin retention in which heparin induced conformational changes of CTX A3 lead to citrate-mediated dimerization. A bound citrate ion stabilizes hydrophobic contact of homodimer at the functionally important regions. The heparin hexasaccharide interacts with 5 CTX A3 molecules in the crystal structure, providing another mechanism whereby the toxin establishes a complex network of interactions that result in a strong interaction with cell surfaces presenting heparin. Our results suggest a novel role for venom citrate in biological activity and reveal a model that explains cell retention of CTX A3 through HS-CTX interaction. The combined usage of the SPR method and the retention test provides a new approach to address the biological function of protein-HS interaction near the membrane surface. The systematic design of the suitable heparin for crystallization and using sulfur SAD signal of heparin for modeling will also be discussed.

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