MOLECULAR RECOGNITION OF CELL SURFACE GLYCOSAMINOGLYCANS BY EOSINOPHIL CATIONIC PROTEIN. STRUCTURAL CLUES FOR ITS CYTOTOXIC ACTIVITY

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Eosinophil Cationic Protein (ECP) is a highly stable cytotoxic RNase of 133 aminoacids present in large amounts in the eosinophil granules. In inflammatory processes, following eosinophil activation the protein is secreted and plays a role in host defence with bactericidal, antiviral, and antiparasitic activities. Remarkably, a large number of Arg residues in the protein surface confers a high cationicity and have been related to ECP's potent cytotoxicity by facilitating the interaction with membranes[1]. Aromatic residues have also been shown to importantly contribute to this activity[1]. These residues have also been reported to be essential for the interaction with glycosaminoglycans (GAGs), such as heparin[2], suggesting that recognition of heparan sulphates exposed at the mammalian cellular surfaces may drive and modulate its cytotoxicity.

To get deeper insight into the cytotoxic process, here we further our previous work[3] and explore the interactions of ECP with a GAG mimetic. By using NMR spectroscopy and molecular dynamic simulations we have determined the solution structure of ECP in complex with a representative trisaccharide heparin-derivative as a good model for the highly sulphated S-domains of heparan sulphate. We have also estimated its binding affinity (μM range) on the basis of chemical shift perturbation data. Filtered experiments were useful to define the orientation of the carbohydrate in the binding pocket, which was found to occupy the catalytic site.

The complex structure reveals that the charged sulphate and carboxylate groups of the carbohydrate are clustered at the binding site with well-defined orientations with respect to the protein favouring electrostatic interactions to be established. Charged and polar residues, as well as the conserved aromatic residue W10 play essential roles in the recognition. Interestingly, the skew-boat $^2S_0$ conformation for the pyranose ring of IdoA is clearly preferred as observed in other complexes[4]. This recognition event may constitute the first step of the ECP's cytotoxic mechanism of action by facilitating contacts with the membrane that would then trigger membrane destabilization and cell death.

[1]Carreras, E.; Boix, E.; Rosenberg, H. F.; Cuchillo, C. M.; and Nogués, M. V. Both aromatic and cationic residues contribute to the membrane-lytic and bactericidal activity of eosinophil cationic protein. *Biochemistry* **2003**, 42, 6636-6644.

[2]Fan, T.-C.; Fang, S.-L.; Hwang, C.-S.; Hsu, C.-Y.; Lu, X.-A.; Hung, S.-C.; Lin, S.-C.; and Chang, M. D.-T. Characterization of molecular interactions between eosinophil cationic protein and heparin. *J. Biol. Chem.* **2008**, 283, 25468-25474.

[3]García-Mayoral, M. F.; Moussaoui, M.; de la Torre, B. G.; Andreu, D.; Boix, E.; Nogués, M. V.; Rico, M.; Laurents, D. V.; and Bruix, M. NMR structural determinants of eosinophil cationic protein binding to membrane and heparin mimetics. *Biophys. J.* **2010**, 11, 2702-2711.

[4]Nieto, L.; Canales. A.; Giménez-Gallego, G.; Nieto, P. M.; Jiménez-Barbero, J. Conformational selection of the AGA*IA_M heparin pentasaccharide when bound to the fibroblast growht factor receptor. *Chem. Eur. J.* **2011**, 17, 11204-11209.