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Dual recognition of lipids and saccharides employing a single binding site in a pore forming toxin

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Fragaceatoxin C (FraC) is a pore forming toxin (PFT) that interacts with biological membranes using a lipid binding motif. Since FraC also interact with the carbohydrates, we investigated the basis of glycan recognition. Surprisingly, we found that FraC engages glycans using the same, high-affinity, lipid-binding module. In conclusion, FraC has developed dual recognition capabilities with a single binding motif.

1 Introduction

PFTs comprise a heterogeneous family of toxic proteins that target foreign cellular membranes with offensive (or defensive) purposes. For example, human perforin is a protein of defensive purposes of the immune system, whereas bacterial PFTs are potent virulence factors causing cell death.

FraC is a small but very potent hemolytic PFT of ~20 kDa secreted by sea anemones, lethal to small marine organisms. Membrane selectivity is achieved by its affinity for the lipid sphingomyelin (SM) of the membrane, which acts as a binding receptor [1]. Interestingly, FraC experience a significant delay in size-exclusion chromatography (SEC) due to a supposedly interaction with the carbohydrate matrix of the resin [2]. However, the putative binding site for carbohydrates or their effect on the function and structure of FraC are still unknown.

2 Experiment

A solution of microcrystals was prepared like that described for unbound FraC [1]. Large rod-shaped single crystals of the complex of FraC and GlcNAc(6S) were obtained after mixing 1 µl of protein at 9 mg ml^{-1} in 10 mM Tris–HCl and 50 mM GlcNAc(6S) (pH 7.4), 0.2 ml solution of micro-seeds, and 1 µl of crystallization solution composed of 19% PEG 3350, 200 mM ammonium chloride and 100 mM Bis–Tris (pH 6.3). Suitable crystals appeared after one month at 20°C. Crystals were subsequently frozen in the presence of 20% glycerol by immersion in liquid nitrogen. Data collection was carried out at beamline AR-NW12A of the Photon Factory (Tsukuba, Japan).

3 Results and Discussion

In this study we hypothesized that the single-domain hemolysin FraC could recognize lipids and carbohydrates by a mechanism different from that of more complex multi-domain PFTs. We thus investigated the ability of FraC to interact with carbohydrates from various points of view. We first employed carbohydrate arrays to identify a population of saccharides containing a negative charge for which FraC showed preferential binding. We thus characterized the binding site of the sulfated monosaccharide GlcNAc(6S) by X-ray crystallography at high resolution (1.5 Å). Surprisingly, the carbohydrate pocket overlapped with the lipid-binding module of FraC (Fig. 1). The binding was characterized by low affinity and no remarkable specificity, suggesting that there could be another, more specific glycan from marine animals, not included in the human glycan array used for the screening step. In summary, we reveal a novel recognition mechanism by an ancient PFT that could enhance the activity of FraC in the natural marine environment [3].

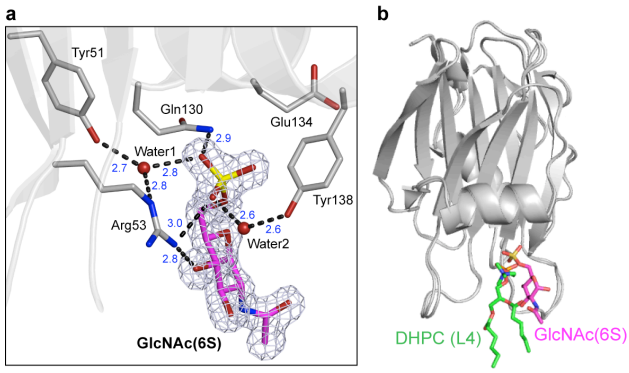

Fig. 1: Crystal structure of FraC in complex with the monosaccharide GlcNAc(6S). **(a)** Sigma-A weighted 2fo-fc electron density map contoured at 1.5 σ. **(b)** Comparison of the relative position of the lipid (green) and saccharide (magenta) in the bound conformation.

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References

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