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Crystal structure of IgG-Fc bound to aglycosylated FcγRIIIa.

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Antibody-dependent cell-mediated cytotoxicity (ADCC) is a key element to mount an effective immune response. ADCC is mediated by the binding of the Fc region of IgG to the cell-surface receptor FcγRIIIa. Herein we have investigated the influence of the carbohydrates (glycans) attached to FcγRIIIa for the interaction with IgG-Fc by determining the crystal structure of the complex when the receptor is in the aglycosylated state.

1 Introduction

Therapeutic antibodies of the IgG class are a critical tool to combat severe diseases including cancer. These class of therapeutic molecules have greatly expanded over the past few decades. A key element for the proper functioning of IgG is the glycan attached to residue Asn297 of the Fc region [1,2]. This glycan not only stabilizes the structure of the antibody, but also modulates key effector functions such as ADCC. In ADCC, the Fc region of IgG binds to the cell surface receptor FcγRIIIa eliciting the cell-response. The composition of the glycan, and in particular the presence of a fucose moiety, influences the strength of the ACDD response thorough interactions with the glycan moiety also present in the receptor. To help clarifying the molecular basis of this interaction we have determined the crystal structure of IgG-Fc bound to aglycosylated FcγRIIIa.

2 Experiment

FcγRIIIa with a His_6 tag at the C-terminus was expressed in *Escherichia coli*. Cells expressing the receptor were harvested and lysed by sonication. The protein was purified by Ni^{2+}-affinity chromatography followed by size exclusion chromatography. The Fc fragment of the therapeutic antibody Rituximab (Roche) was obtained by papain digestion and purified with a Protein A kit. The purified IgG-Fc was mixed with purified Fcγand the complex further purified by size exclusion chromatography in 20 mM Tris-HCl, 100 mM NaCl at pH 7.4. Fractions containing the complex were concentrated to 5.0 mg/mL. Crystallization was carried out with an Oryx8 instrument (Douglas Instruments) followed by rounds of manual optimization. Single crystals were obtained in a solution containing 14% PEG 3,350 and 100 mM NaCl by the hanging drop method.

Suitable crystals were harvested and frozen in the presence of 25% glycerol. Data collection was carried out at beamline BL5A of the Photon Factory (Tsukuba, Japan) at 100 K. The structure was determined by the method of molecular replacement using the coordinates of the complex of the glycosylated receptor (PDB Id 3AY4). The coordinates of the complex have been deposited in the protein data bank with accession code (5YC5).

3 Results and Discussion

The crystal structure of the complex between IgG-Fc and FcγRIIIa was determined at 2.7 Å (Fig. 1). No significant differences in the architecture of the receptor or the antibody were observed. This result demonstrates that the glycan present in FcγRIIIa is not critical for the binding conformation of the complex with IgG-Fc. Instead it suggests that the glycan moieties influence the dynamic behavior of the complex.

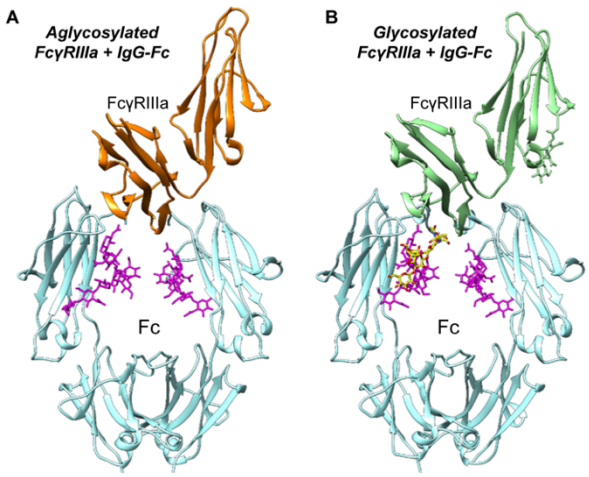

Fig. 1: Crystal structure of IgG-Fc in complex with **(a)** aglycosylated or **(b)** glycosylated FcγRIIIa [3].

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