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Crystal structure of the ultra-high affinity complex between CFH and a nanobody

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The crystal structure of a VHH in complex with complement factor H (CFH) was determined at 2.60 Å resolution revealing the molecular basis of the ultra-high affinity of the VHH for CFH (picomolar order). The epitope is located in the Leu1181–Leu1189 loop of CFH. This observation is significant, because this loop is recognized by autoantibodies causing atypical hemolytic uremic syndrome, a condition related to the dysregulation of the complement system.

1 Introduction

The disease termed atypical hemolytic uremic syndrome (aHUS) is characterized by hemolytic anemia and acute kidney injury, as well as thrombocytopenia [1]. Genetic and acquired defects causing dysregulation of the complement system are associated with this disease. In this respect, the protein complement factor H (CFH) downregulates the alternative pathway and it is considered one of the causes for aHUS. CFH is a large protein but only the C-terminal domains (CCP18-20) are involved in binding to C3b of the complement system.

In this study, an anti-CFH antibody of the VHH class showing ultra-high affinity was obtained, and its structure in complex with domains CCP18-20 of CFH determined at 2.60 Å resolution [2].

2 Experiment

The protein CFH18-20 was expressed in Expi293 cells and purified to homogeneity. VHH4 was expressed in *Escherichia coli* BL21 cells and similarly purified to homogeneity. Single crystals of the complex between CFH18-20 and VHH were obtained by the method of sitting drop in a solution of 200 mM sodium phosphate monobasic monohydrate and 20% w/v polyethylene glycol 3350 at 20 °C using an Oryx 8 instrument (Douglas, UK). Single crystals were harvested and frozen in liquid N_2. Data was collected in beamline BL5A of the Photon Factory (Tsukuba).

3 Results and Discussion

Diffraction of the crystals prepared from above were collected and processed to 2.60 Å resolution, and refined (Table 1). The structure revealed the molecular basis of the ultra-high affinity of the VHH for CFH. Coincidentally the epitope of this VHH coincides with that of autoantibodies related to the atypical hemolytic uremic syndrome. This structure provides significant insights to help to rationalize how autoantibodies recognize CFH and cause disease.

Table 1: Data collection and refinement statistics.

Data Collection	**CFH18-20 - VHH**
Space Group	P 32 22 1
Unit cell	
a, b, c (Å)	75.96, 75.96, 143.20
Angles (°)	$\alpha = \beta = 90; \gamma = 120$
Resolution (Å)	48.4 – 2.60 (2.74 – 7.60)
Unique reflections	15,303 (2,204)
Rmerge	0.16 (1.26)
$R_{p.i.m.}$	0.045 (0.35)
$I / \sigma (I)$	12.3 (2.6)
Multiplicity	13.5 (13.6)
Completeness (%)	99.9 (99.8)
Refinement	
Resolution (Å)	48.4 – 2.60
R_{work} / R_{free} (%)	19.1 / 23.9
No. atoms	
CFH	1,018
VHH	973
Water	25
B-factor (Å2)	
CFH	70.5
VHH	66.2
Water	54.6
Ramachandran Plot	
Outliers (%)	0

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References

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