Viral and human membrane glycoproteins

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Results

1. Glycoprotein D from herpes simplex virus I

During last trips at the Biocars C beam line data were collected from two different crystal forms of glycoprotein D (HSV-1):

- Crystal form A (Spcgr. P4(2)2(1)2 with a = b =158 Å and c = 154 Å);
- Crystal form B (Spcgr. P4(x)2(x)2 with a = b = 132Å and c = 83 Å).

A native (2.8 Å resolution / Rsym 6.6% [29% in the last resolution shell] / 98.5% complete) and a few heavy atom soaks have been collected from crystal form I. Several small crystals (0.100 x 0.025 x 0.025 mm^3) of crystal form II have been tested to access their diffraction properties. Crystal form II presents some degree of layer disorder that manifests in elongation of the reflections in the a* and b* directions. Data from four native crystals (3.5 Å resolution) have been collected and the data are now under evaluation.

2. Complex between gD and the virus's human receptor protein HveC

Two data sets have been collected from gD-Hvec crystals. The data from one of them extend to 4.0 Å (Rsym = 7.2% [22% in the last resolution shell] / 99.8% complete). The crystals belong to the space group P3(2)21. Analysis of the intensity distribution indicates the crystals might be merohedrally twinned (twinning operator -h, -k, l / the estimated twinning fraction [using statistical method of Yeates] is between 0.34 and 0.5 depending on the crystal).

3. Influenza virus haemagglutinins of new subtypes from recent virus outbreaks.

We collected native data sets from H3 (A/Dk/Ukr/63), H5 (A/Dk/Sing/97), H7 (A/Eq/Pra/56), and H9 (A/Sw/HK/99) subtype haemagglutinin crystals. H5 and H9 caused outbreaks in Hong Kong; the former (H5) resulted in a number of deaths. Currently, why the virus did not spread into the general population is unknown. We have also collected diffraction data from complexes of H3, H5, and H9 haemagglutinins with two receptor analog pentasaccharides, Lsta and Lstc, to study the change in receptor specificity that accompanies influenza moving from animals and birds into humans.

The H9 structure was determined with MIRAS and H5 and H7 structures were solved with molecular replacement using the H9 model as the probe. The H3 structure was solved also by molecular replacement using a human H3 (X31) model. We found that H5, H7, and H9 type structures differ from the H3 type structure in their globular head domains by a rotation of about 20 degrees relative to the central fibrous domains. Residues at positions 75 and 88 of the HA2 chain (on which the head domain sit) are the key to the structural difference. We conclude that of the 15 haemagglutinin subtypes, H3, H4, and H14 belong to one structural group while the rest belong to the other. From the complex structures with LSTa and LSTc, we learned that the second sugar of the pantasacchride adopts a "face-on" conformation when the 226 position is a Leu, and adopts a "flat" conformation when residue 226 is a Gln, which may lead to an understanding of receptor, and therefore, host specificity.

4. Three-dimensional structure of a complex between human T cell receptor HA1.7, influenza virus haemagglutinin peptide HA 306-318 and human MHC class II protein HLA-DR1

The purpose of this project was to get more structural insight into the specific recognition of antigen peptide/MHC class II complexes by T-cell receptors.

A crystal of the human MHC class II protein HLA-DR1 and the influenza virus haemagglutinin peptide HA 306-318 in complex with their cognate T cell receptor HA1.7 has been grown in 12% PEG8000, 1 M NaCl, 100 mM HEPES (pH = 7.5) to a size of 200 x 100 x 30 μm and frozen using 20% glycerol as a cryoprotectant. The complex crystallized in spacegroup C2 with cell dimensions of a = 142.9 Å, b = 3.4 Å, c = 122.4 Å, and β = 108.2º. Data of this crystal have been measured on the BioCars C-station (400 images, 30 second exposure time, 0.75º oscillation per image). Processing of the data with DENZO and SCALEPACK gave a native dataset with the following statistical values:

Resolution (Å)	25.0–2.6 (2.69–2.60)
Mosaicity (deg.)	0.75
No. unique reflections	37159
Multiplicity	6.0 (4.7)
Completeness	99.7 (99.8)
Average I/σI	17.7 (7.9)
R_{merge} (%)	7.6 (22.1)

The dataset has been used to determine the structure of the complex by molecular replacement. The structure has been refined and is currently being analyzed and prepared for publication.