## 03-Crystallography of Biological Macromolecules



**PS-03.02.12** STRUCTURAL STUDY OF BLUTONGUE VIRUS (BTV) CAPSID PROTEIN VP7. By A. K. Basak , J. Grimes*, P. Roy and D. I. Stuart. Laboratory of Molecular Biophysics, Oxford University, U. K.

Blutongue virus (BTV) belongs to the Orbivirus genus of the Reoviridae family and has a double capsid structure. It infects livestock and wild ruminants. Twenty four different serotypes of the virus have been identified from different parts of the world. The BTV genome cosists of seven structural proteins (VP1-VP7) and three non-structural proteins (NS1-NS3). The icosahedral core of BTV, which has a diameter of some 700Å , consists of five proteins, two major proteins (VP3 and VP7) and three minor proteins (VP1, VP4 and VP6). This core is surrounded by an outer capsid made from two proteins, VP2 and VP5. BTV-VP7 forms the bulk of the core with 780 copies arranged on a T=13 lattice.
The protein has been synthesized in the insect cells infected with a recombinant baculovirus AcBTV-10.7 containing the VP7 gene of serotype 10. Two crystal forms suitable for X-ray analysis have been grown.

| form | a | b | c | angle | difrc$^n$ | Sp. group |
|---|---|---|---|---|---|---|
| I | 95.2Å | 95.2Å | 181.0Å | $\gamma$=120° | 3.0Å | $P6_322$ |
| II | 69.4Å | 97.1Å | 71.4Å | $\beta$=109.3° | 2.1Å | $P2_1$ |

A self-rotation function and dynamic light scattering studies, both confirmed that VP7 forms a trimer in both crystal forms and also in solution.
The hexagonal crystal form has been solved to a resolution of 6Å spacing. From an SIR, solvent flattened map, calculated using a AuCN derivative, it is clear that VP7 is a two domain protein forming a tripod type structure.
Unfortunately, the monoclinic form is composed of the upper domain only, with two trimers in the asymmetric unit. This structure has been solved to a resolution 2.4Å using a single derivative and solvent flattening to 4.0Å, followed by phase extension to 2.4Å using the 6-fold non-crystallographic redundancy in the asymmetric unit. The real space averaging was done using the GAP programme suite (Stuart, D. I. and Grimes, J.M. unpublished).
The upper domain is an anti-parallel $\beta$-sandwich that bears strong similarity to the jelly roll motif common to most viral capsid proteins. The intimate trimeric association is mediated by two extended loops from each monomer. One loop interacts with both of its trimeric partners at the top of the molecule forming a tight annulus. The second loop runs inside the trimer just below the top loop, interacting with its symmetry related partners and the top loop, before turning and running down to form the last strand of the back sheet of a three fold related subunit. These loops are very tightly packed with the trimer being locked together by one loop clipping onto the $\beta$-sandwich of an adjacent subunit. VP7 is structurally most similar to the jelly roll of haemagglutanin from influenza virus.

### 03.03 - Immunoproteins and Growth Factors

**MS-03.03.01** 2.8 Å RESOLUTION STRUCTURE OF AN IDIOTYPE-ANTIIDIOTYPE FAB COMPLEX. STEPHEN V. EVANS, REBECCA TO, DAVID R. ROSE, N. MARTIN YOUNG, AND DAVID R. BUNDLE, INSTITUTE FOR BIOLOGICAL SCIENCES, NATIONAL RESEARCH COUNCIL OF CANADA, OTTAWA, CANADA, K1A 0R6.

The complex formed between Fab YsT9.1, specific to anti-*Brucella abortus* cell wall polysaccarides, and its antiidiotypic Fab T91AJ5 has been crystallized and data have been collected to 2.8 Å resolution in the orthorhombic space group $P2_1$ with $a$ = 51.49, $b$ = 141.71, $c$ = 79.53 Å, with $\beta$ = 98.9°. The structure was solved by molecular replacement, and has been refined to a current R-value of 0.184. This is the first reported structurally characterized example of an idiotype-antiidiotype complex where the original idiotype was raised against a polysaccharide antigen. The complex is a head-to-head dimer, with the contact between both Fabs completely restricted to their hypervariable loops. However, the antiidiotype has not been observed to mimic the original antigen in that it cannot be used to raise 'anti-antiidiotypes' which bind the original polysaccharide. The final three-dimensional structure determination provides a molecular basis for this observed lack of mimicry.

**MS-03.03.02** SHAPE COMPLEMENTARITY AT ANTIBODY-ANTIGEN INTERFACES. By M.C. Lawrence* and P.M. Colman, Biomolecular Research Institute, 343 Royal Parade, Parkville, Victoria 3052, Australia.

It is recognized that the binding interfaces of antibody/antigen complexes display high levels of shape complementarity (Davies *et al.* Annu. Rev. Biochem., 1990, 59, 439-473). Such intimacy of association excludes most if not all water molecules from the interface. However no systematic attempt has been made to compare quantitatively the complementarity and packing of antibody/antigen interfaces with those of oligomeric and protein/inhibitor interfaces. Results have been confusing, suggesting either that antibody/antigen interfaces are less well packed (Tulip *et al.*, J. Mol. Biol., 1992, 227, 122-148) or contrarily that the packing is similar to that found in protein interiors (Walls and Sternberg, J. Mol. Biol., 1992, 228, 277-297). We have examined, using a novel statistic termed the *median normal complementarity* N, the surface complementarity of all antibody/antigen complexes deposited in the Protein Data Bank, plus a selection of oligomeric and protein/inhibitor complexes. N has advantages over other measures of interface packing in that it does not require assignment of atomic volumes and is relatively insensitive to the definition of the molecular surface. Results for the above selection of interfaces will be presented.

**MS-03.03.03** THE STRUCTURE OF CD2, A CELL ADHESION MOLECULE By E. Y. Jones*, S. J. Davis, A. F. Williams, K. Harlos and D. I. Stuart. Laboratory of Molecular Biophysics, Oxford University, U. K.

The immunoglobulin superfamily molecule CD2 plays a key part in mediating adhesion between T lymphocytes and accessory or target cells.
Expression of the soluble, extracellular region of rat CD2 (sCD2) in a lectin-resistant mutant of the Chinese hamster ovary cell line, Lec3.2.8.1 resulted in a secreted glycoprotein with oligosaccharides that were amenable to deglycosylation. The deglycosylated sCD2 crystallized in space group $P4_12_12$ with unit cell dimensions a = b = 111.4, c = 86.9Å and two molecules per asymmetric unit (60% crystal solvent content). Multiple isomorphous replacement, phase improvement and extension by solvent flattening and, finally, a single round of 2-fold density averaging, yielded unambiguous electron density. The resultant two models of sCD2 have been refined in the program XPLOR to a current R factor of 21.5% for all data to 2.8Å with good stereochemistry and non crystallographic restraints.
The crystal structure of rat CD2 provides the first complete view of the extracellular region of a cell adhesion molecule. The topology of the molecule, which comprises two immunoglobulin-like domains, is the same as that of the first two domains of CD4 but the relative domain orientation is altered by a fairly flexible linker region. The putative ligand-binding $\beta$-sheet forms a flat surface towards the top of the molecule. Crystal contacts between these surfaces suggest a plausible model for the adhesive interaction.

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