ESRF	**Experiment title:** Structure determination of cV_HE2 antibody domain, a macromolecular inhibitor of the HCV NS3 protease	**Experiment number**: **LS1803**
Beamline: ID14-2	**Date of experiment**: from 5-10-2000 to 6-10-2000 (12 hours)	**Date of report**: 15-07-01
Shifts to BAG: 3	**Local contact(s)**: Julien Lescar	*Received at ESRF:*

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One of the most studied targets for HCV therapy is the serine proteinase activity of NS3 protein of HCV. A macromolecular inhibitor of the N-terminal serine protease of the HCV NS3 protein has been affinity selected from a library constructed by inserting randomized oligonucleotides in the region corresponding to the CDR3 of prototype camelized human variable domain antibody fragment (see F. Martin et al. *Protein Engineering* (1997) **10**, 607-614 for details). This inhibitor has a Ki of 150nM. In analytical gel filtration experiments the molecule appears to behave as a dimer, although it is unknown whether it is functionally active as a dimer or a monomer. The macromolecular inhibitor cV_HE2 has been purified and crystallized and X-ray data were collected at ESRF (Table 1). The 'camelised' cVHE2 domain structure was solved by molecular replacement using the Lama Glama Heavy chain variable domain, against alpha-subunit of Human chorionic Gonadotropin (PDB entry code, 1HCV), which shares a sequence identity of 69%. The crystal structure was solved and refined at 1.8 Å (Table 1 and fig.1).The protein is a dimer in the crystal, which was not yet observed in human or mouse heavy variable domains. The overall shape of the molecule is displayed as a ribbon diagram of the backbone in fig.2. The architecture of the cVHE2 as anticipated fits the well-known variable domain immunoglobulin fold with a four-stranded β–sheet facing a five-stranded β -sheet. (Chothia, et al, 1988; Padlan et al 1994).

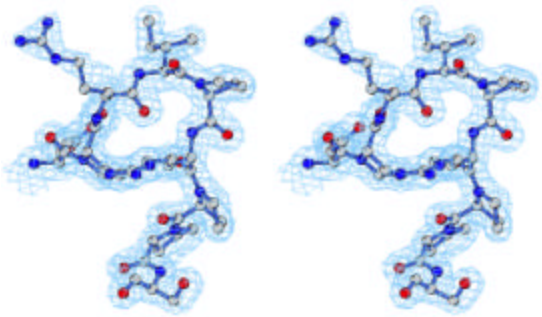

Fig. 1, Stereo diagram of the 2Fo-Fc electron-density map of the cV_HE2 (residues 99 to 107) at 1.8 Å resolution, as illustration of the quality of the map. The map is contoured at 1σ.

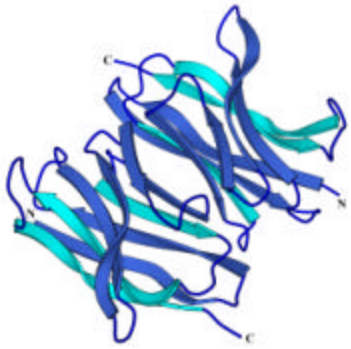

Fig. 2, Ribbon diagram of cVHE2 dimer. β-strands are in blue and sky-blue, loops are in blue. Colours are not related to structure features.

Interestingly the major change noted in the structure is the dimeric crystal packing arrangement in which the two homodimers are packed head to tail. This association is unlike the arrangement of VL and VH in a FV fragment. Contrary to a FV, none of the three CDRs of these two domain participates in the interdomain contacts in the homodimer structure. Moreover this association places the two VH domains in a relative spatial arrangement corresponding to a quasi heterodimeric FV structure where one of the two domains has been rotated 180° in respect to the other. The β-strand structure of the two homodimers can be superimposed with a r.m.s. of 0.2 Å. A possible inhibitor binding mechanism for the cVHE2 to the NS3 active site is explained (Fig.3).

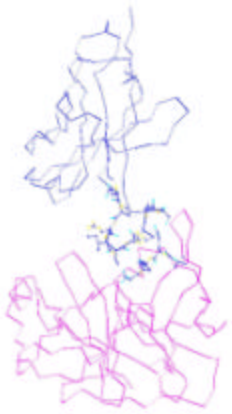

Fig. 3, Cα model showing the hypothetical binding of the cVHE2 to the NS3 protease active site cVHE2 monomer is drawn in blue and NS3 protease in magenta, the CDR3 is shown in ball and stick as the inhibitor bound to the protease.

The more favorable interaction between the antigen and NS3 seems to happen at the top of the NS3 active site. It is not possible to position the CDR3 directly in the active site pocket of NS3, in the same binding position of the inhibitor, because this will cause an intermolecular clash between the cVHE2 and NS3, suggesting the existence of only one way for interfering with the catalytic activity. Under this hypothesis the CDR3 would determine a sterical hindrance, not allowng in this way a simultaneous substrate and inhibitor entrance, so explaining what previously reported by us about the competitive nature of the cVHE2 (Martin et al, 1997).

Table 1

Crystallographic Data collection and refinement statistics

Unit cell parameters (Å)	a = b = 75.30 c = 101.78 α = β = 90 γ = 120
Space group	P3(2)21
Resolution range (Å)	30 – 1.8
No. reflections measured	69,437
No. unique reflections	29,910
completeness (%)	94.9 (71.5)[a]
R_{merge}[b](%)	5.1 (22.7)
$\langle I \rangle / \langle sI \rangle$	17.2 (2.4)
B_{wilson} ($Å_2$)	19.3
R-factor (%)[a]	19.20
Free *R*-factor (%)[b]	25.64
No. of protein atoms	1850
No. of solvent atoms	399
rms deviations from ideal geometry	
bond distance (1-2) (Å)	0.019
angle distance (1-3)	0.038
planar distance (1-4)	0.044
Chiral volumes ($Å^3$)	0.16
Planar torsion angles (deg.)	4.9
Deviations from planarity (peptides) (Å)	0.03
Deviations from planarity (aromatics) (Å)	0.01
mean B-factors ($Å^2$)	
all atoms	23.79
protein atoms	20.34
Glycerol (2)	41.77
Sulphate ions	49.14
Water molecules	37.71
Ramachandran plot statistics	
residues in most favoured regions (%)	93.6
residues in additional allowed regions (%)	6.4
residues in disallowed regions (%)	0

[a] Values in parentheses are for the highest resolution shell (1.84 – 1.80 Å). [b] $R_{merge} = \sum_{hkl}\sum_{i=1}^{N}\left|\langle I^{hkl}\rangle - I_i^{hkl}\right| \Big/ \sum_{hkl}\sum_{i=1}^{N} I_i^{hkl}$

$R = 100\sum_h \| F_o - F_c \| / \sum_h |F_o|$

[b] Free *R*-factor is calculated from 5%of the data which were omitted during course of the refinement

A paper is going to be submitted soon.

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