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- 1st March for experiments carried out up until June of the previous year;
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Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** X-ray crystallography of viral proteins and gramicidin S analogues	**Experiment number**: MX-363
Beamline: ID23-1	**Date of experiment**: from: 27-September-2004 to: 28-September-2004	**Date of report**: 20-October-2005
Shifts: 3	**Local contact(s)**: Dr Davi Richard Hall	*Received at ESRF:*

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Report:

Introduction

Avian reovirus causes serious disease in birds, leading to important losses in the poultry industry. Nevertheless, not much is is known about their biochemistry and structure. Ten structural proteins have been identified in strain S1133: core components lambdaA, lambdaB, lambdaC, muA and sigmaA and external proteins muB, muBC, muBN, sigmaB and sigmaC. Four non-structural proteins are also encoded: p10, p17, sigmaNS and muNS. Avian reovirus is highly resistant to the antiviral effects of interferon and the dsRNA-binding sigmaA protein apparently plays an important role in that resistance. The sigmaA protein is capable of reversing the interferon-induced antiviral state by down-regulating PKR activity in a manner similar to other virus-encoded dsRNA-binding proteins. The avian reovirus cell-attachment sigmaC protein is a trimer, but the receptor to which it binds is unknown.

The Det7 bacteriophage belongs to the Myoviridae bacteriophage family (although it is not completely T4-like), but has an enzymatically active tail protein related to the P22 bacteriophage tailspike (Podoviridae family). It infects the bacteria Salmonella enteritidis, which is an important pathogen.

Gramicidins are non-ribosomally synthesized peptides. Gramicidin S (GramS), produced by Bacillus brevis, is a cationic cyclic deca-peptide antibiotic with twofold symmetry: cyclo-[Val-Leu-Orn-DPhe-Pro]2. It has antimicrobial activity against bacteria and fungi. Our collaborators, Gijs Grotenbreg and Mark Overhand of the Bio-Organic Synthesis group (Leiden University, the Netherlands) have synthesised native GramS and various analogues in order to study the influence of sugar amino acid residues on the peptide structure and antibiotic activity. One of the analogues (although with low biological activity) has already been crystallised and its structure solved. In its structure, six GramS analogue molecules form a beta-barrel with a hydrophobic exterior and hydrophilic interior, indicating GramS may function as a membrane channel. We have crystallised the native form and two more sugar amino acid analogues. We have also crystallised a F-phenyl-derivative GramS synthesised in the group of Anne Ulrich, of which 19F-solid state NMR studies show that gramicidin S tilts at high concentration. This would bring the charges into the middle of the bilayer

and it was proposed that oligomerisation into a beta-barrel is a way of avoiding charges in the middle of bilayer.

Results obtained

SigmaA

We have measured native data on several crystals of sigmaA to around 2.5 Å. The spacegroup appears to be P1, with cell parameters of 103 x 130 x 144 Å and angles of alpha = 94º, beta = 105º, gamma = 98º, meaning the asymmetric unit probably contains many copies (between 6 and 15). Furthermore, all crystals show multiple lattices, therefore we have up to now not been able to solve the structure by molecular replacement. We are currently trying to improve the crystals on one hand and obtain heavy atom derivatives and seleno-methionine derivatives on the other.

SigmaC

Avian reovirus fibre, a homo-trimer of the sigmaC protein, is responsible for primary host cell attachment. The protein expressed in bacteria forms elongated fibres comprised of a carboxy-terminal globular head domain and a slender shaft, and partial proteolysis yielded a carboxy-terminal protease-stable domain that was amenable to crystallisation. We tested a crystal grown in the same condition as measured on BM16 just before, at ID23-1. It yielded nice data to around 3.0 Å. The space-group was $P6_322$ with cell dimension 76 x 76 x 243 Å. We were not able to solve the structure by molecular replacement using the mammalian reovirus fibre sigma1, but have later obtained a different crystal form (P321, cell of 75 x 75 x 74 Å), which diffracted to higher resolution, and solved the structure of that crystal form using the MAD technique on a mercury derivative. The work has been published in the following two papers:

M. J. van Raaij, X. L. Hermo Parrado, P. Guardado Calvo, G. C. Fox, A. L. Llamas-Saiz, C. Costas, J. Martínez-Costas and J. Benavente: Crystallisation of the C-terminal globular domain of avian reovirus fibre, *Acta Cryst.* F61, 651-654 (2005).

P. Guardado Calvo, G. C. Fox, X. L. Hermo Parrado, A. L. Llamas-Saiz, C. Costas, J. Martínez-Costas, J. Benavente and M. J. van Raaij: Structure of the carboxy-terminal receptor-binding domain of avian reovirus fibre sigmaC, J. Mol. Biol., in press.

The carboxy-terminal globular domain has a beta-barrel fold with the same overall topology as the mammalian reovirus fibre (sigma1). However, the monomers of the sigmaC trimer show a more splayed-out arrangement than in the sigma1 structure. Also resolved are two triple beta-spiral repeats of the shaft or stalk domain. The presence in the sequence of heptad repeats amino-terminal to these triple beta-spiral repeats suggests that the unresolved portion of the shaft domain contains a triple alpha-helical coiled-coil structure.

Crystallographic data and refinement statistics

Beam-line (ESRF)	ID23-1
Detector (MAR-CCD)	Mosaic 225 mm
Wavelength (Å)	0.97565
Space group	$P6_322$
Cell parameters (a,b / c, Å)	75.60 / 243.14
Mosaic spread (º)	0.78
Resolution range (Å)	30-3.0 (3.2-3.0)
Observed reflections[c]	8895 (1256)
Multiplicity	8.8 (9.2)
Completeness (%)	99.8 (100.0)
Rsym[d] (%)	7.7 (26.2)
<I/sigma(I)>	8.2 (2.9)
Rejected measurements (%)[e]	1.06
Resolution range used, refinement (Å)	30-3.0 (3.1-3.0)
No. of reflections used, refinement	8003 (556)
No. of reflections used, R-free	885 (70)

R-factor[f]	0.198 (0.27)
R-free	0.249 (0.34)
No. of protein / water atoms	1260 / 44
Average B-value protein/solvent (Å^2)	44.6 / 35.9
Ramachandran statistics[g] (%)	84.8/12.4/2.1/0.7
R.m.s. deviations[h] (bonds, Å/angles, °)	0.014 / 1.7

[a]Ångström (0.1 nm).
[b]Values in parentheses are for the highest resolution bin, where applicable.
[c]No sigma cut-off was used for inclusion of observed reflections.
[d]$R_{sym} = \Sigma_h \Sigma_i |I_{hi} - \langle I_h \rangle| / \Sigma_h \Sigma_i | I_{hi} |$, where I_{hi} is the intensity of the i^{th} measurement of the same reflection and $\langle I_h \rangle$ is the mean observed intensity for that reflection.
[e]Measurements with intensities differing more than 3.5 standard deviations from the mean were rejected.
[f]$R = \Sigma||Fobs(hkl)| - |Fcalc(hkl)|| / \Sigma|Fobs(hkl)|$.
[g]According to the program PROCHECK. The percentages are indicated of residues in the most favoured, additionally allowed, generously allowed and disallowed regions of the Ramachandran plot, respectively.
[h]Estimates provided by the program REFMAC.

The bacterophage Det7 tailspike

Double-stranded DNA bacteriophage can be divided into several families, of which *Myoviridae*, T-even and similar phages, are one, and *Podoviridae*, T7-like phages, are another. The two families have a morphologically different tail. *Myoviridae* have a long, contractile tail with a base-plate, to which long and short tail fibres are attached. *Podoviridae* have a short base-plate directly attached to the neck of the phage and lack a contractile tail and long tail fibres. To this base-plate, six tailspikes are normally attached. The best-studied podovirus tailspike is that of bacteriophage P22. It is very stable parallel homo-trimer and its structure can be divided into three domains: an amino-terminal head-binding domain, a central beta-helix domain containing 13 complete beta-helix turns and a carboxy-terminal, partially intertwined, domain postulated to be important for pro-trimer formation. The amino-terminal domain is flexibly attached to the other two, which together form a rigid unit; the flexibility is thought to be important in the infection process. The central beta-helix domain binds to the bacterial host cell and is involved in hydrolysis of O-antigen. The O-antigen hydrolysis function of tailspike is thought to be too slow for being important in the fast infection step. Promoting lateral movement on the bacterial surface also does not seem a necessary step. Rather, the hydrolysis function is perhaps important for escaping bacterial debris after cell lysis.

A new bacteriophage, Det7, discovered in sewage (Regensburg, Germany), has morphology and sequences characteristic of myoviruses, but a tailspike with 50 % overall sequence identity to the podovirus P22 tailspike. In collaboration with Dr. Stefan Miller (Profos AG, Regensburg, Germany), we have crystallised the Det7 tailspike without the amino-terminal phage-binding domain and, after optimisation of the crystallisation conditions, obtained large crystals of hexagonal shape. At ID23-1 these crystals diffracted x-rays to 1.6 Å, spacegroup R32, cell 100 x 100 x 329 Å (hexagonal settings). We solves its structure using the structure of the podovirus P22 tailspike (pdb-code 1TSP) on the beamline and later refined it at home.

The bacteriophage Det7 tail tailspike is a parallel homo-trimer, virtually identical to the bacteriophage P22 tailspike. The structure contains an amino-terminal three-helix bundle (which connects to the absent phage-binding domain), a central beta-helical domain and a carboxy-terminal intertwined domain. Trimer contacts are both hydrophilic and hydrophobic, as in the case of the P22 tailspike.

The central domain is composed of three beta-helical monomers, each left-handed beta-helix being composed of 13 complete turns. The beta-helices have a hydrophobic core and interact with each other through polar interactions. The "dorsal fin", consisting of residues 242-304, is inserted in the fourth helical turn, and the sixth, seventh, eighth, ninth and tenth helical turn also have insertions. The dorsal fin flanks one side of the receptor-binding groove, the other insertions flank the other side. Residues reported to contact the O-antigen in the P22 tailspike are well-conserved: the active site residues are all identical, and in the other contacting side-chains there are only two differences, Trp365 of P22 is Tyr410 in Det7, and Ser237 of P22 is Asn282 in the Det7 tailspike sequence. Det7 tailspike binds octasaccharide somewhat less tightly than P22 tailspike; this could be due to Trp365 in the P22 tailspike active site being a Tyr in the Det7 tailspike. The

structure of the carboxy-terminal domain, with its interdigitating and extensive intersubunit contacts, suggests that folding may start with this domain, as has been proposed for the P22 tailspike.

The Det7 tailspike is, after the P22 tailspike and the Shigella phage Sf6 tailspike, the third known tailspike structure. For P22 a transient disulphide bridge between Cys613 and Cys635 of a neighbouring monomer in the trimer has been proposed on the folding pathway and mutating the two cysteines to serine blocks tailspike assembly. The tailspikes of phages Det7 and Sf6 do not contain the cysteines involved, so they may have a different folding pathway. **A manuscript entitled "Structure of the receptor-binding protein of bacteriophage Det7, a myovirus with a podovirus tailspike" is in preparation to be submitted.**

GramicidinS analogues

We measured datasets of various gramS analogues, of which we were able to solve the structure of one, containing a naphtylated sugar amino acid as a replacement of one of the two Pro-D-Phe beta-turn regions. The others either did not diffract to high enough resolution, or had too many atoms in the asymmetric unit to allow structure solution by direct methods. The peptide adopts a beta-sheet conformation featuring an unusual reverse turn induced by the sugar amino acid. The altered turn region induces a distortion of the antiparallel beta-sheet, as compared to native gramS, the overall geometry however closely resembes that of the non-naphtylated gramS analogue we had solved previously. This structure has been described in the following paper, which has been submitted for publication: **G. M. Grotenbreg, A. E. M. Buizert, A. L. Llamas-Saiz, M. J. van Raaij, G. A. van der Marel, H. S. Overkleeft, M. Overhand et al.: Beta-turn modified gramicidin S analogues containing arylated sugar amino acids are as potent as the parent compound.**

Acknowledgements

M.J.v.R. is supported by an “Ramón y Cajal” contract of the Spanish Ministery of Education and Science. This work was supported by the following grants: BMC2002-02436 (Spanish Ministry of Education and Science) and PGIDIT03PXIC20307PN (Xunta de Galicia). The European Union provided 70% of grant BMC2002-02436 through the FEDER programme.