

Ref. No.: 2010-6
Title: Foot-and-mouth disease virus assembly: Effect of myristoylation and proteolytic processing on the biological properties of pentameric capsid subunits
IAH Supervisors: Dr Toby Tuthill and Dr Terry Jackson
Programme: Foot-and-Mouth Disease
Location: Pirbright Laboratory
Funder: BBSRC/IAH
University: Imperial College, London
University Supervisor: Professor Stephen Curry

Project Abstract

Foot-and-mouth disease virus (FMDV) is a member of the picornavirus family of small, single-stranded, positive-sense RNA viruses with a non-enveloped icosahedral capsid, comprising 60 copies each of 4 structural proteins VP1-4. Assembly proceeds from a monomeric polyprotein-precursor (VP0, VP1 and VP3), via a pentameric intermediate, to the complete capsid. On encapsidation of the viral RNA genome, the capsid undergoes a final maturation cleavage of VP0 to produce VP2 and VP4, which is myristoylated at its N terminus. The capsid plays a number of important functions; it has to protect the viral RNA genome but needs to be sufficiently flexible to allow for genome release during cell-entry. The capsid also interacts with specific cell-surface receptors to allow attachment to target cells and is recognised by antibodies during the immune response to infection. We hypothesise that myristoylation and presence of VP4 are critical for correct assembly of pentameric capsid subunits and for their biological properties.

We have recently demonstrated that recombinant capsid precursors have virus-like antigenicity and receptor binding properties and can be used to study pentamer assembly. After cleavage by the viral protease 3Cpro, myristoylated precursor assembled into pentamers whereas unmyristoylated precursors assembled into novel aberrant structures, while precursors that lack VP4 did not undergo assembly. This project will build on these findings by investigating the role of myristoylation on the structure, stability, antigenicity and receptor binding of capsid pentamers and the potential role of VP4 as a conformational 'switch' that controls pentamer receptor binding and antigenicity. This is an exciting area of research as it will reveal novel insight to FMDV capsid assembly and stability and provide important information for the rational design of more stable vaccines for the control of this important disease.

Full description of project

1 Introduction

FMDV is highly contagious and has devastating economic consequences worldwide. The 2001 UK outbreak resulted in the slaughter of 4 million animals and cost the economy £10

billion. FMDV is the type species of the genus Aphthovirus of the family Picornaviridae, which are small, single-stranded, positive-sense RNA viruses with a non-enveloped icosahedral capsid. The structure of FMDV has been solved to high resolution by X-ray crystallography. The positive-sense RNA genome contains a single open reading frame that is translated into a large polyprotein from which a smaller structural-region polyprotein is derived by co-translational processing. This becomes myristoylated at its N-terminus and acts as the capsid precursor, the monomeric building block from which new virus particles are made. The monomer is characterised by its rate of sedimentation of 5S in sucrose density gradients. Further post-translational processing is mediated by the FMDV protease 3Cpro, which allows five processed monomers to associate into a pentameric assembly intermediate (pentamer) which sediments at ~12S. Twelve pentamers assemble to form the complete capsid which packages a single molecule of genomic RNA. On RNA encapsidation, the capsid undergoes a maturation cleavage of VP0 into VP2 and VP4, to produce infectious virus.

All of the capsid components associated with the assembly pathway (monomer, pentamer, capsid) contain VP4 and share important antigenic features. However, during cell entry the FMDV capsid dissociates at low pH, into pentamers and releases VP4. These dissociated pentamers have significantly altered antigenicity and this is likely to be because they no longer contain the VP4 component. VP4 may therefore be a switch that controls the conformation and antigenicity of virus capsid components. Understanding these processes would provide novel insights to FMDV capsid assembly and provide important information of benefit to the design of improved vaccines.

2 Recent findings leading to the project

Our recent studies with recombinant proteins (Goodwin et. al. 2009) have demonstrated that the antigenicity and receptor binding properties of capsid precursor are dependent on both the polyprotein composition and myristoylation. We have also shown that after cleavage by viral protease 3Cpro, myristoylated capsid precursors assembled into pentamers with an expected sedimentation (12S) while unmyristoylated precursors instead assembled into novel structures with altered sedimentation (17S) and precursor that lacks VP4 did not assemble.

3 Aims

To determine the role of myristoylation in the structure, stability, antigenicity and receptor binding of the pentamer and to investigate the proposed role of VP4 as a conformational switch that controls pentamer receptor binding and antigenicity.

4 Experimental procedures

4.1 Effects of myristoylation: Are recombinant myristoylated 12S pentamers equivalent to native pentamers? What is the 17S form of recombinant unmyristoylated 'pentamer'? What are the properties of incompletely myristoylated pentamers? Sedimentation, antigenicity and receptor binding properties of pentamers will be compared using established procedures for ultracentrifugation and ELISA. The stability and appearance of 17S structures will be investigated by electron microscopy and multimeric status will be determined by chemical crosslinking and size determination by SDS-PAGE, in comparison with 12S material. Preliminary data shows that unmyristoylated radiolabelled monomers can be incorporated into

12S pentamers. What proportion of unmyristoylated monomer can be tolerated by the 12S pentamer (e.g. 1 in 5?), does incorporation influence pentamer properties?

4.2 Effects of specific polyprotein cleavages: What are the requirements for pentamer assembly? We and others have shown that in order for assembly to proceed, capsid precursor protein requires processing by viral protease 3Cpro (cleavage at VP0/VP3 and VP3/VP1 junctions). We will now investigate whether one or both cleavages are critical, by analysing the effect of engineered cleavage site knock-outs on the assembly and properties of pentamers. In order to mimic the effect of the VP0 maturation cleavage in the pentamer, a unique cleavage site specific for an exogenous protease will be introduced between VP4/2 and the release of VP4 and alteration of pentamer properties investigated with methods described above.

4.3 Production of reagents: Expression of recombinant proteins and analysis of pentamer assembly will use methods established in the laboratory. Native pentamers containing VP4 (assembly pathway) will be purified from infected cells. Pentamers lacking VP4 (dissociation pathway) will be produced by acid treatment of virus and gradient purification.

4.4 Capsid assembly: dependent on progress, the project may extend to investigate the self-assembly and stability of empty capsids, using fluorescent and structural biology approaches.

Research activity of the Group

Toby Tuthill joined the Institute for Animal Health in 2009 with nine years post-doctoral experience working with picornaviruses at the University of Leeds. Research interests include virus structure, entry, capsid assembly and genome packaging, use of liposomes as model membranes to study virus-membrane interactions.

References to project for further reading

1. Goodwin S, Tuthill TJ, Arias A, Killington RA, Rowlands DJ. Foot-and-mouth disease virus assembly: processing of recombinant capsid precursor by exogenous protease induces self-assembly of pentamers in vitro in a myristoylation-dependent manner. *J Virol.* 2009 Aug 26. [Epub ahead of print]
2. Moscufo N, Chow M. Myristate-protein interactions in poliovirus: interactions of VP4 threonine 28 contribute to the structural conformation of assembly intermediates and the stability of assembled virions. *J Virol.* 1992 Dec; 66(12):6849-57.
3. Sweeney TR, Roqué-Rosell N, Birtley JR, Leatherbarrow RJ, Curry S. Structural and mutagenic analysis of foot-and-mouth disease virus 3C protease reveals the role of the beta-ribbon in proteolysis. *J Virol.* 2007 Jan;81(1):115-24.

4. Curry S, Fry E, Blakemore W, Abu-Ghazaleh R, Jackson T, King A, Lea S, Newman J, Stuart D. Dissecting the roles of VP0 cleavage and RNA packaging in picornavirus capsid stabilization: the structure of empty capsids of foot-and-mouth disease virus. *J Virol.* 1997 Dec;71(12) :9743-52.