Expression of the Foot and Mouth Disease Virus Protease in *E. coli*

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1 Introduction

The coding sequences of the genome of foot and mouth disease virus (FMDV) form a continuous open reading frame of about 7 kb, which is translated into a single polyprotein of about 250,000 M.W. It is processed post-translationally by the action of a virus-encoded protease and possibly also host-specific proteases (for a review see Sangar 1979).

Understanding of this process requires elucidation of the role the virus protease plays in maturation of FMDV proteins. We therefore established a system in *E. coli* cells (Klump et al. 1984) that (1) separates the virus protease activity from interfering host-specific protease activities and (2) is suitable for protein processing.

By this means proteins were produced which range in size from 45,000 to 180,000 M.W. and have identical NH₂-terminal amino acid sequences specific for the capsid protein VP1. Their COOH-termini differ according to the different length of FMDV cDNA which is expressed. The polyprotein fragments produced in *E. coli* exhibit some cleavage sites which are identical to those present in the polyprotein produced in virus-infected BHK-cells (Sangar 1979). Analogous to the processing of the viral precursors in BHK-cells, the virus-specific proteins expressed in *E. coli* should be cleaved by the virus-encoded protease if it is expressed in *E. coli* in an active form.

2 Plasmid Constructions

2.1 The Expression System, Construction of pPLVP1

*E. coli* clones have been described which carry FMDV O₁K-specific cDNA (Küpper et al. 1981; Fig. 1A). In the same report subcloning of the gene encoding the capsid protein VP1 (BamHI-HindIII cDNA fragment of clone 1034, see Fig. 1A) into the vector pPlc24 (Remaut et al. 1981), which enables the expression of VP1 in *E. coli*, has been described. In this construction designated pPLVP1 the FMDV reading frame is in phase with that of the 5'-adjacent gene fragment of phage MS2 replicase, which has all signals necessary for initiation of translation. Transcription depends on the leftward promoter pr of bacteriophage λ, which is controlled by the λ repressor cI857, a temperature-sensitive mutant repressing at 28 °C but not at 42°C. Hence vector-dependent expression is induced by shifting the temperature of the bacterial cultures from 28°C to 42°C.

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The HindIII-EcoRI cDNA fragment of clone 715 and the EcoRI-HindIII cDNA fragment of clone 703 (Fig. 1A) were inserted into pPLVP1 at the HindIII site adjacent to the BamHI-HindIII cDNA fragment. The recombinant plasmid contains the FMDV genes encoding VP1 and the precursors P52 and P56c (Fig. 1B,C).