The Amino-acid Sequence at the Carboxyl Terminus of the Maturation Protein of Bacteriophage MS2

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Amino-acid sequence at the carboxyl terminus of the phage MS2 maturation protein provides direct evidence for the nucleotide sequence and reading frame proposed in the preceding communication.

THE amino-acid sequence of proteins coded by RNA phage genomes is of great interest, as it is technically possible to determine the nucleotide sequences and so directly compare gene products with gene structure[1]. Remaut and Fiers[2] have obtained evidence that the maturation protein terminates with a single UAG stop codon. Contreras *et al.*[3] determined the sequence of nucleotides preceding the initiating AUG of the coat protein and deduced that an UAG, located twenty-six nucleotides before the former AUG, was the termination signal of the preceding A cistron. By determining the amino-acid sequence of the carboxyl end of the A protein, we can provide direct proof for this conclusion.

Bacteriophage MS2 was prepared and purified in 10 g batches from 100 l. enriched medium cultures[4]. The A protein was isolated according to Osborn *et al.*[4] and further purified by gel filtration on 'Sephadex G-200' in buffer containing 0.1% sodium dodecyl sulphate (SDS). Excess SDS was removed by dialysis and by precipitation of the protein with ethanol from a formic acid-phenol solution.

The amino-acid at the carboxyl terminus was identified as arginine by hydrazinolysis (ref. 5 and Hilschmann, personal communication) and by digestion of the protein by successive treatment with carboxypeptidase B and A in the presence of 0.1% SDS[6].

Chymotryptic digestion was performed after reduction, carboxymethylation and citraconylation[7]. Decitraconylation was obtained by keeping the digest overnight at *p*H 3.5 and 37° C. A first separation of the peptides, dissolved in 5% acetic acid, was obtained by gel infiltration in 'Sephadex G-25'. The peptide mixtures in the different fractions were further purified by paper and thin layer chromatography. The amino-acid composition of each peptide was determined with a 'Biocal 201' analyser. The amino-acid sequence of the chymotryptic peptides was determined by the Edman-Dansyl procedure (ref. 8 and R. Perham, personal communication).

Trypsin digestion was carried out on the reduced and amino-ethylated protein. Peptides soluble in 10% acetic acid were purified as indicated above.

On comparison with the nucleotide sequence determined by Contreras *et al.* we identified five chymotryptic and four of the five expected tryptic peptides. On the basis of this nucleotide sequence we can order these peptides as represented in Fig. 1. Our results confirm the reading frame proposed by these authors and show conclusively that the suggested UAG is the stop codon of the maturation protein gene.

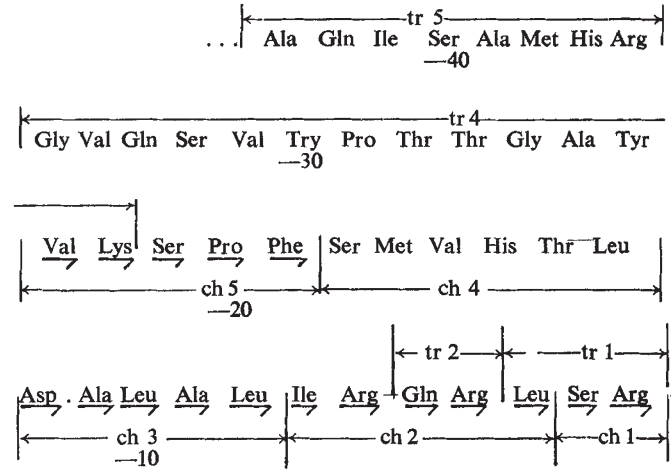

Fig. 1 Amino-acid sequence of the 43 C-terminal amino-acids of the maturation protein. The chymotryptic peptides ch 1 to 5 and the tryptic peptides tr 1, 2, 4 and 5 are indicated. The small arrows indicate the stepwise modified Edman degradations performed.

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