TRANSLATION OF MENGOVIRUS RNA IN EHRLICH ASCITES CELL EXTRACTS

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SUMMARY

Mengovirus RNA was translated in Ehrlich ascites cell extracts using as radioactive precursors f[^{35}S]Met $tRNA_F^{Met}$ and [^{35}S]Met $tRNA_M^{Met}$ to label the products in N-terminal and internal positions, respectively. Tryptic peptides were compared with those derived from purified [^{35}S]Met-labeled mengovirus. The results indicate that the sequences corresponding to the viral coat polypeptides are preceded by a short "lead-in" peptide which is probably removed by a cleavage process in infected cells.

INTRODUCTION

The synthesis of some proteins in eukaryotes apparently starts with a short "lead-in" peptide (1-3). This peptide is subsequently cleaved off to yield either a final product in the case of immunoglobulin light chain (1), or a polypeptide that is processed further by post-translational cleavage in the case of encephalomyocarditis (EMC) virus proteins (2,3). The cleavage mechanism is probably not active in cell-free translating systems where the synthesis of the precursor polypeptide has been observed (1-3). It is not clear if the cleavage of the short peptide is different from post-translational processing of picornavirus polyprotein (4-6) or if the lead-in sequence has some special function (1). In this communication we report another example , the translation of mengovirus RNA, where the cleavage of a lead-in sequence is indicated.

MATERIALS AND METHODS

[^{35}S]Met tRNAs were prepared and purified as described earlier (2), and their purity was determined by paper electrophoresis (7). The contamination of fMet $tRNA_F^{Met}$ by unformylated Met $tRNA_F^{Met}$ was less than 3%. Preparation of [^{35}S]Met-labeled mengovirus, mengovirus RNA and S10 or S30 extracts from Ehrlich ascites

cells have been described (8). After trypsin digestion (8), the peptides were dissolved in 1 ml of starting buffer a, which includes per liter: 10 ml of pyridine, 15 ml of N-ethylmorpholine, 20 ml of α-picoline, and acetic acid to pH 9.4. After the sample had been adjusted to pH 10, it was applied to a 1 x 60 cm column of AGI-X2 (Biorad) 200-400 mesh resin. Elution at 35° C (flow rate = 30 ml/hr) was performed with 75 ml buffer a, followed consecutively by linear gradients of the following composition: b = 150 ml buffer a plus 150 ml buffer a adjusted to pH 6.4 with acetic acid; c = 150 ml buffer a at pH 6.4 plus 150 ml 1 M pyridine-acetic acid, pH 5.1; d = 150 ml 1 M pyridine-acetic acid, pH 5.1 plus 150 ml 2 M pyridine-acetic acid pH 5.1 and e = 150 ml of 2 M pyridine-acetic acid, pH 5.1 plus 150 ml acetic acid. Fractions of 3-3.5 ml were collected and counted in Triton-toluene scintillation mixture. Recovery was better than 85%. Other methods have been described earlier (2).

RESULTS AND DISCUSSION

As has been shown earlier, translation in vitro of EMC, mouse Elberfeld and mengovirus RNA starts at one initiation site giving rise to one predominant fMet-labeled tryptic peptide when the radioisotope is f[^{35}S]Met $tRNA_F^{Met}$ (2). The elution position of the mengovirus N-terminal tryptic peptide from an anion exchange column is shown in Fig. 1. Edman degradation (2) reveals that the label in this peak is 91% terminal (Table 1). When the in vitro translation of mengovirus RNA is done in the presence of [^{35}S]Met $tRNA_M^{Met}$, the resulting labeled tryptic peptides can be separated as shown in Fig. 2. It should be noticed that the major peak labeled with [^{35}S]Met $tRNA_M^{Met}$ coincides with that obtained with f[^{35}S]Met $tRNA_F^{Met}$, eluting at about fraction 220 at 1.3 M pyridine-acetic acid, pH 5.1. The amount of terminal label in the tryptic peptides is, however, only 13% in this fraction and 11% in the peak at fraction 295 (Table 1).

The tryptic digest of [^{35}S]methionine-labeled proteins from purified mengovirus yields the separation pattern shown in Fig. 3. Since virion coat protein sequences account for less than 50% of the coding capacity of the picornavirus genome (9), some of the tryptic peptides found in the in vitro products are

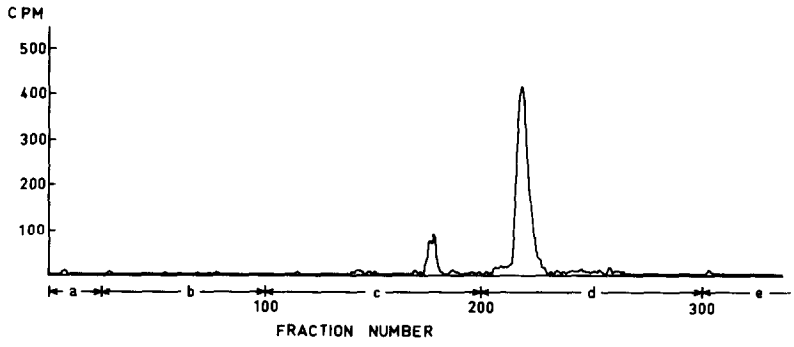

Figure 1. Anion exchange chromatography of tryptic digest of f[^{35}S]Met-labeled mengovirus proteins synthesized in vitro. The method has been described earlier (2).

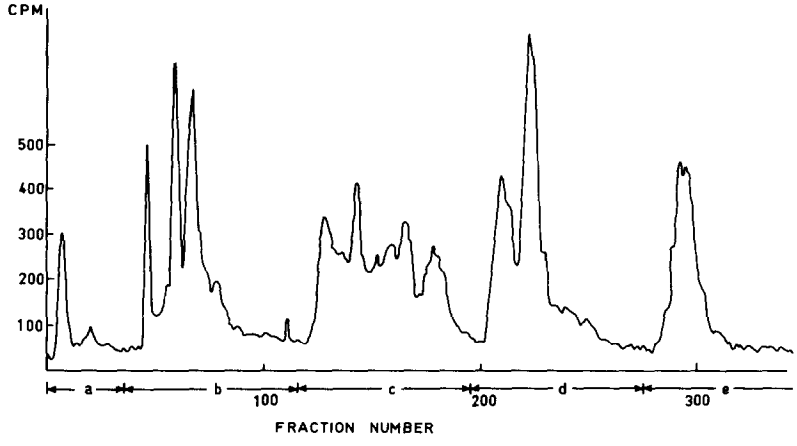

Figure 2. Anion exchange chromatography of tryptic digest of [^{35}S]Met-labeled mengovirus proteins synthesized in vitro as described earlier for EMC virus proteins (2).

absent from the trypsin digest of virions. This is especially pronounced for the peptides eluting at lower pH, i.e. fractions about 210, 220, and 300 (compare Figs. 2 and 3). The virion coat tryptic peptides contain 11% N-terminal methionine by Edman degradation (Table 1).

Premature termination of polypeptide products takes place when EMC virus RNA is translated in ascites cell-free extracts (2,3,8,10,11). This results in a preponderance of capsid peptides, in agreement with the EMC virus genetic map

Table 1. Edman degradation of tryptic peptides

Sample	Terminal counts/min	%	Internal counts/min	%
Tryptic peptides from [^{35}S]methionine labeled mengovirus	5634	10.8	46517	89.2
Fraction 210-230 in Fig. 1	1636	91	155	9
Fraction 205-230 in Fig. 2	66	13	452	87
Fraction 280-310 in Fig. 2	75	11	631	89
Fraction 185-200 in Fig. 3	56	8	680	92

Lyophilized samples from fractions in Fig. 1-3 and unfractionated tryptic digest of [^{35}S]methionine-labeled mengovirus were subjected to Edman degradation and analyzed as described earlier (2).

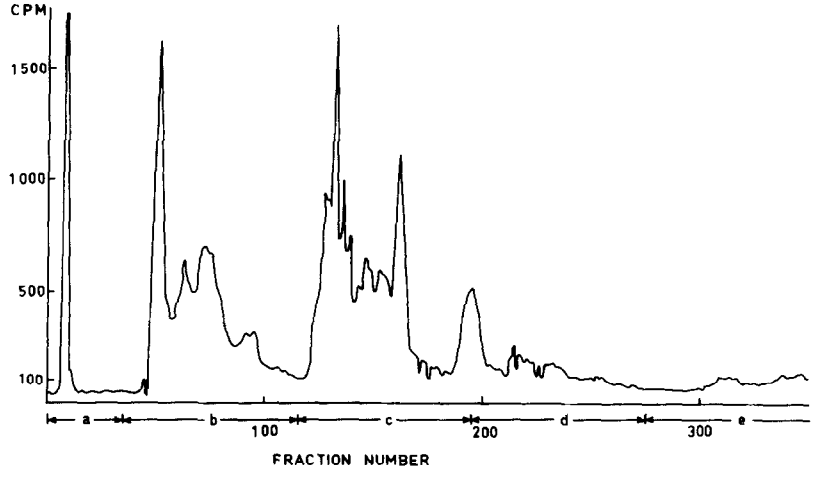

Figure 3. Anion exchange chromatography of tryptic digest of [^{35}S]Met-labeled mengovirus.

which places the coat protein sequences near the 5' end of the viral genome (9). The predominant synthesis of mengovirus capsid peptides *in vitro* (Fig. 2) indicates that they are also coded for by sequences close to the 5' terminus of the viral RNA. The N-terminal tryptic peptide, obtained during labeling with f[^{35}S] Met $tRNA_F^{Met}$ (Fig. 1), contains a formylated methionine which should contribute very little to its elution position. It is likely that it is identical with the tryptic peptide at fraction 222 in Fig. 2, apart from the formylated methionine. In the case of EMC virus RNA translation, the major tryptic peptide from the *in vitro* products also coincided with the tryptic peptide labeled with f[^{35}S] methionine (2), a result expected for prematurely terminated products containing an even distribution of methionine.

The N-terminal peptide obtained from the mengovirus *in vitro* products (Fig. 1) is absent from the virion tryptic peptides (Fig. 3). This result indicates that cleavage of a short peptide precedes the completion of the structural proteins found in mature virions and suggests that coat protein sequences start at a short distance from the N terminus of the viral polyprotein. This was shown previously for EMC virus protein synthesized *in vitro* where the short "lead-in" peptide has a molecular weight of about 2000 (2, 3). Thus, the translation of mengovirus and EMC virus RNA, and perhaps other picornavirus genomes, appears to be very similar.

It has been reported that immunoglobulin light chain synthesized *in vitro* also contains at the N terminus a peptide of about 1500 daltons which is removed *in vivo* by cleavage (1). It was suggested that the lead-in sequence may be involved in intracellular transport of immunoglobulin (1). However, the function of a short lead-in peptide between the initiation site and the coat sequences in picornavirus proteins remains to be elucidated.

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