The position of polypeptide G on the encephalomyocarditis virus polyprotein cleavage map

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The two-dimensional mapping of tryptic peptides of encephalomyocarditis virus-specific proteins has demonstrated that the amino acid sequence of non-structural polypeptide G constitutes a portion of the molecule of a precursor of capsid proteins, polypeptide A. The results of pulse-chase in vitro translation experiments strongly suggest that polypeptide G corresponds to a C-terminal moiety of polypeptide A. Variations in the polyprotein cleavage maps of different picornaviruses are briefly discussed.

Picornavirus *Encephalomyocarditis virus* *Translation* *Post-translational processing* *Polyprotein cleavage map*

1. INTRODUCTION

Genomic RNA of picornaviruses has a single site for initiation of translation and directs the synthesis of giant polyprotein molecules which undergo multiple proteolytic cleavages before being ultimately converted into 'mature' virus-specific polypeptides. The schemes that reflect the precursor–product relationships of polypeptides formed upon the processing of the polyprotein as well as the order, in this polyprotein molecule, of the amino acid sequences corresponding to particular polypeptides are termed polyprotein cleavage maps. The general outline of the cleavage maps of different picornaviruses was elucidated about a decade ago (review [1]), and many important details have been learned since. The polyprotein of encephalomyocarditis (EMC) virus, while in a nascent form, is cleaved into leader polypeptides [2] and polypeptides A, F and C (in this order from the N-terminus) [3]. Polypeptide A is a precursor of the capsid proteins, polypeptide F is a stable product with yet undefined functions, and polypeptide C is converted into a number of proteins that include participants of the viral genome replication as well as the viral protease [3–5]. However, the map position of several low-M_r polypeptides remained uncertain. Among these was polypeptide G, a basic RNA-binding protein [6,7] which was found in association with ribosomes of the virus-infected cells [8].

Mapping polypeptide G by following the incorporation of labelled amino acids into different proteins after the arrest of initiation of translation suggested that G should be placed somewhere between the capsid proteins and polypeptide F [9], although other possibilities were also considered by the same investigators [9,10]. There was a proposal that G, like A, was a product of the primary cleavage of the nascent polyprotein [9]. In [7] we observed that G was produced upon the treatment with the viral protease of a mixture of high-M_r polypeptides formed upon a short incubation of an EMC virus RNA-programmed cell-free system. Since these high-M_r polypeptides consisted largely of precursors of the capsid proteins, it might be suggested that a capsid protein precursor contained the amino acid sequence of G. Such conclusion, however, could be challenged inasmuch as the substrate for protease was not precisely

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characterized in those experiments.

Here, we will show that all the methionine-containing tryptic peptides of G can be found in the molecule of polypeptide A, a precursor of the capsid proteins. Pulse-chase in vitro translation experiments suggest that G corresponds to a C-terminal portion of A.

2. METHODS

The infection of Krebs-II cells with EMC virus was done as in [11]. The in vivo labelling of virus-specific proteins was achieved by incubation of the infected cells with [^{35}S]methionine (1200 Ci/mmol; 50 μCi/ml) from 3.5–4.5 h. Viral RNA was translated in micrococcal nuclease-treated extracts from uninfected Krebs-II cells [4]. As substrates, [^{35}S]methionine (50 Ci/mmol; 100 μCi/ml) or a mixture of ^{14}C-labelled lysine, 354 Ci/mol, leucine, 348 Ci/mol and valine, 265 Ci/mol (Amersham), each at 5 μM, were used in experiments aimed at obtaining the proteins for fingerprinting and in kinetic studies, respectively.

Electrophoretic separation of the virus-specific polypeptides was done in 12.5% or 8–20% gradient polyacrylamide gel slabs in the presence of

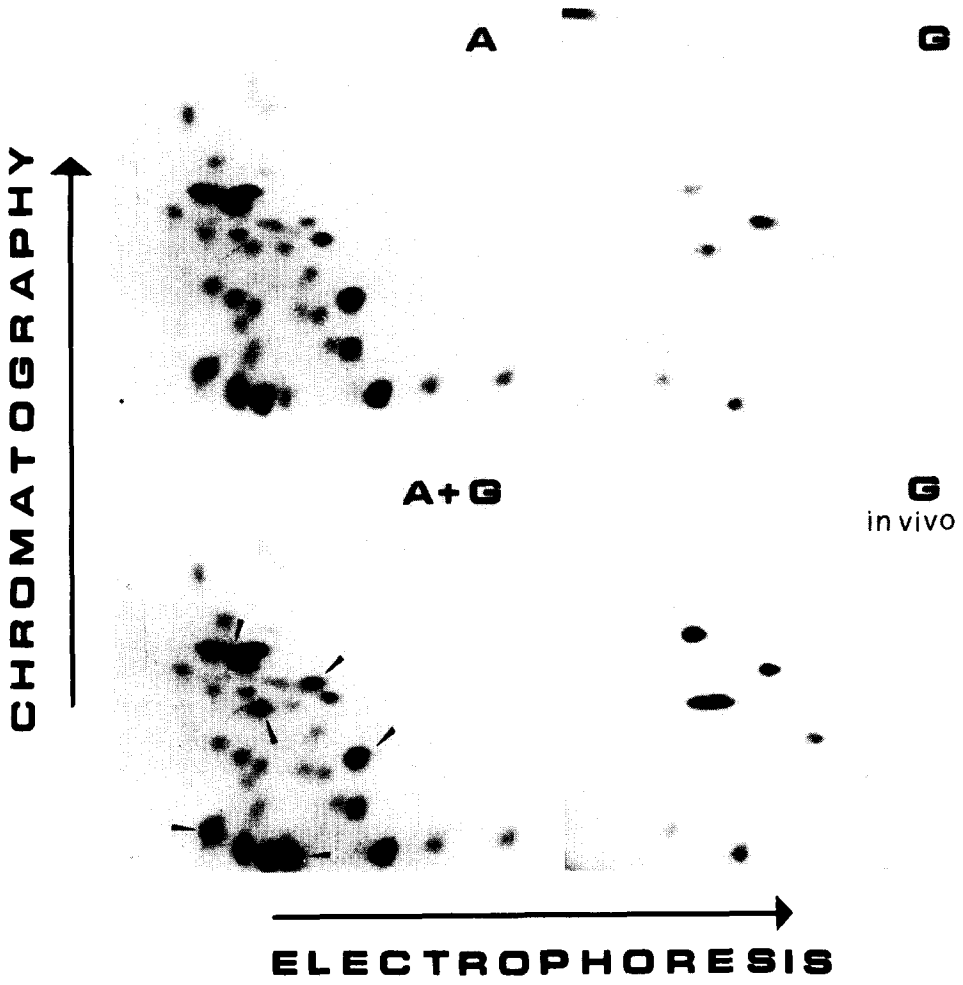

Fig.1. A comparison of tryptic peptide maps of polypeptides A and G. The polypeptides were isolated either from the products of cell-free synthesis (two upper photographs), or from the virus-infected cells (G in vivo) by preparative electrophoresis in 12.5% polyacrylamide slab gels. A map obtained upon photographic superimposition of the two upper maps is also given (A + G); ⟶ peptides shared by A and G.

SDS using a discontinuous buffer system [12].

Trypsinolysis and two-dimensional fingerprinting of ^{35}S-labelled polypeptides were performed essentially as in [2].

The kinetics of accumulation of virus-specific polypeptides in vitro was investigated in pulse-chase experiments (see fig.3). The autoradiographs of the electrophoretically separated proteins were scanned using an Acta CIII spectrophotometer (Beckman). The areas under the peaks of radioactivity (expressed in arbitrary units) were divided by the M_r-values of the corresponding polypeptides, and the values of relative radioactivity thus obtained were plotted against the duration of the pulse with radioactive substrate.

3. RESULTS AND DISCUSSION

Circumstantial evidence that the amino acid sequence of polypeptide G was a part of the molecule of a capsid protein precursor was obtained in [7]. To verify this directly, fingerprints of tryptic peptides of A and G were compared. All the methionine-containing peptides derived from G (either synthesized in vitro or isolated from the virus-infected cells) have counterparts in the fingerprint of A (fig.1). Thus, it may be concluded that G is a product of the proteolytic cleavage of A.

Other stable products formed upon processing of A include capsid polypeptides δ, β, γ and α [3].

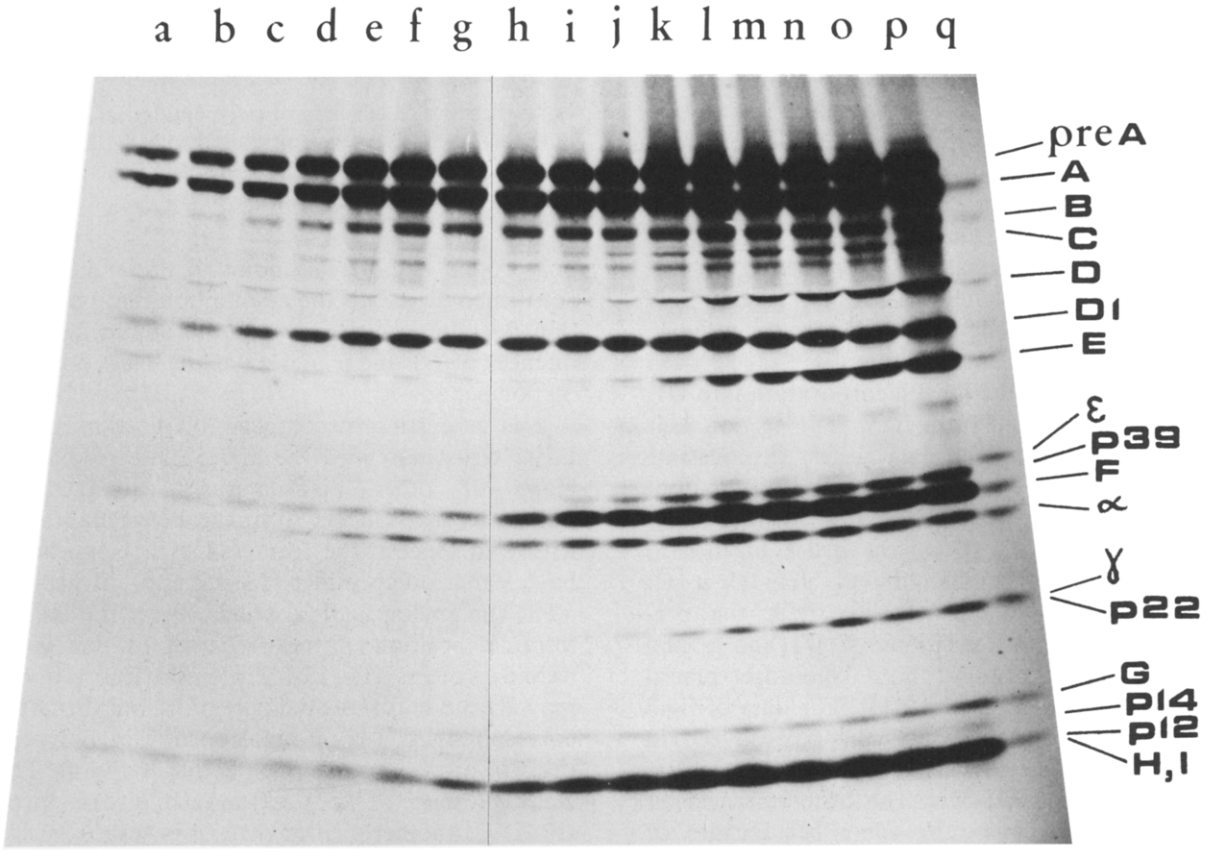

Fig.2. Accumulation of virus-specific polypeptides in a cell-free pulse-chase experiment. Translation samples programmed with EMC virus RNA were incubated with a mixture of ^{14}C-labelled amino acids for (min): 5 (a), 10 (b), 15 (c), 20 (d), 25 (e), 30 (f), 35 (g), 40 (h), 50 (j), 55 (k), 60 (l), 65 (m), 70 (n), 90 (o); cold amino acids in 200-fold excess as well as calf liver tRNA, 130 μg/ml (Boehringer) were then added to each sample, and incubation was continued; the total incubation time (pulse + chase) was 2.5 h. Polypeptides from the sample incubated with labelled amino acids for 2.5 h are shown in lane p, and polypeptides labelled in vivo are shown in lane q. Electrophoresis was performed in an 8–20% gradient polyacrylamide slab gel.

Polypeptides δ, β and γ are known to derive from a common intermediate precursor, polypeptide D1. To determine the position of the amino acid sequence of G relative to the other products of the processing of A, the kinetics of accumulation of polypeptides D1, α and G was studied in in vitro pulse-chase experiments. In these experiments, the total duration of incubation (pulse with labelled substrate + chase with excess cold amino acids) was sufficiently long (2.5 h) to permit both the synthesis of the viral protease and the protease-mediated processing of A into D1, α and G. It may be noted that further processing of D1 proceeded less efficiently in the cell-free system and therefore could not invalidate our conclusions. The electrophoretic patterns obtained in one of such pulse-chase experiments are presented in fig.2. It is seen that the labelled virus-specific polypeptides were accumulating in a certain succession, which should reflect the order of the corresponding amino acid sequences in the polyprotein molecule. The succession did not appear to be appreciably affected by the residual incorporation of the labelled amino acids during the chase period. (This incompleteness of the chase was evidenced by the faint bands of polypeptides C, D, E and F in samples that had been incubated with the radioactive substrates for only 5–10 min.)

The kinetics of the label incorporation into D1, α and G, calculated from fig.2 as in section 2, is presented in fig.3. The data clearly demonstrate that it was the polypeptide D1 moiety of A that was labelled first, then followed the synthesis of α and, finally, of G. Thus, the order of these 3 polypeptides in A should be NH_2-D1–α–G-COOH. This order is in line with the predetermined relative positions of D1 and α and agrees with the proposal that G should be placed somewhere between α and F [3]. The data of fig.3 also suggest that G might probably be produced, in our system, in a relatively lower molar concentration compared to D1 or α. This observation might be an artifact caused by some peculiarities of amino acid composition of the 3 polypeptides; alternatively, it might be explained by premature terminations of translation within the genome region encoding polypeptide A, or by multiple reinitiations of translation.

We can now propose the following cleavage map of the N-terminal portion of the EMC virus

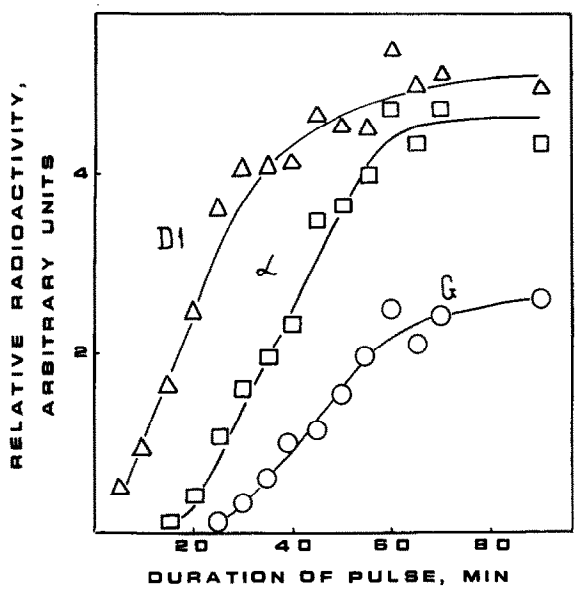

Fig.3. Kinetics of labelling of polypeptides D1, α and G in the pulse-chase experiment presented in fig.2. For the method of calculation of the relative radioactivity, see text.

polyprotein (fig.4). The point in this map to be confirmed directly is the assumption that polypeptide B comprises the sum of the amino acid sequences of D1 and α as well as the exact position of polypeptide I.

It is interesting to compare this portion of the EMC virus map with the appropriate regions of maps of other picornaviruses (fig.4). The poliovirus map differs from the EMC map in at least two aspects: the former does not appear to have leader polypeptides [13–15] and polypeptide VP1, the analogue of α, seems to occupy the C-terminal position of the precursor of poliovirus capsid proteins [16–18]. The poliovirus genome may also code for an analogue of G, but the latter corresponds to a N-terminal portion of polypeptide 3b, a precursor of polypeptide X (fig.4). The cleavage map of foot-and-mouth disease virus (FMDV), another picornavirus, is in some respects intermediate between the EMC virus and poliovirus maps. The FMDV polyprotein, like its EMC virus counterpart, appears to be initiated with leader polypeptides [19], but the FMDV analogue of G, if it exists, originates, most probably, from polypeptide P52, a precursor encoded in the central region of the genome, like in polio

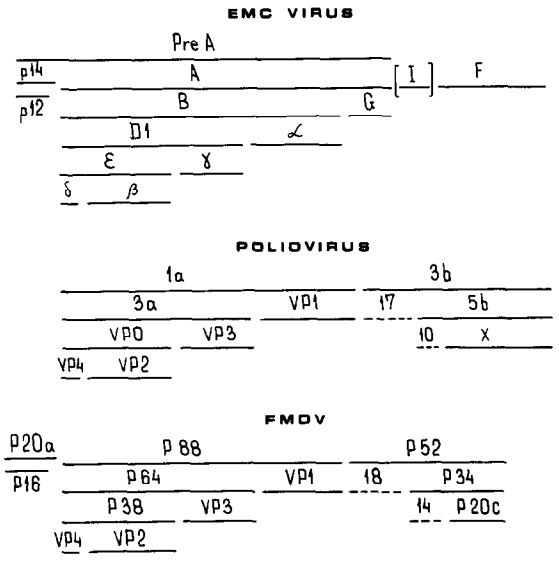

Fig.4. Cleavage maps of the N-terminal regions of the polyproteins of 3 picornaviruses. Data for poliovirus were taken from [13–18] and data for FMDV were taken from [19,20]. The possibility of alternative cleavage of EMC virus polypeptide B into polypeptides ϵ and D2 (as well as homologous polypeptides of other picornaviruses) is not illustrated for the sake of simplicity. Dotted lines represent hypothetical cleavage products. There is no direct evidence that FMDV polypeptides P52, P34, and P20c are actually C-coterminal. The exact position of EMC virus polypeptide I is uncertain.

(fig.4). Thus, despite a remarkably close similarity in the organization of the coding sequences of the 3 picornaviruses, their detailed cleavage maps appear to be different.

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