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Cleavage of the Capsid Protein Precursors of Encephalomyocarditis Virus in Rabbit Reticulocyte Lysates

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The cleavage pathway of the three capsid protein precursors of encephalomyocarditis virus, proteins A1, A, and B, was studied in rabbit reticulocyte lysates. Kinetic data suggested that the three proteins were cleaved through a sequential order of A1 to A to B.

The capsid proteins of encephalomyocarditis virus (EMC), like those of other picornaviruses, are formed from precursor proteins by proteolytic cleavages (1). There are three known EMC capsid precursors: proteins A1, A, and B (3, 4, 5). They are nested polypeptides with the same amino terminus (3). In rabbit reticulocyte lysates, the three precursors are rapidly synthesized, accumulate temporarily, and are then cleaved to the viral capsid proteins by an EMC-coded protease (3, 4). The precursor-product relationship of the three proteins has not been well defined. For example, the order in which A1, A, and B are formed and whether A and B are intermediates in the conversion of A1 to D1 plus α are uncertain. Nor is it clear whether virus-coded or cellular proteases are involved in the synthesis of A1, A, and B. In the present study, we carried out further kinetic analyses of the translation and the cleavage reactions in an attempt to answer these questions.

In the course of studying the translation and processing reactions, we have observed that the rate of peptide chain elongation varied in different reticulocyte lysates. In lysates with slow rates, the time needed for synthesis of protein A1, the largest of the three precursors, was as much as twice that in the 'fastest" lysates. Moreover, in 'slow" lysates, protein A1 accumulated before the formation of significant amounts of proteins A and B and other EMC proteins. Figure 1 shows the formation of EMC proteins as a function of time in one such slow lysate. A strong A1 band was visible after a 25-min incubation. The intensity of the A1 band reached maximum at about 35 min before it started to decline. Proteins A and B appeared as faint bands until about 35 to 40 min, reached maximum at about 50 to 60 min, and then began to decline as the intermediate protein D1 band and capsid protein bands ϵ, α, and γ became prominent. The sequential appearance and disappearance of the three precursors shown here suggest that proteins A and B are intermediates in the conversion of A1 to D1 plus α.

We showed previously that in reticulocyte lysates with rapid translation rates, the three precursor proteins appear at about the same time (at ca. 10 to 15 min of translation) and that they remain unchanged in the lysate if further translation is blocked (4). The appearance of the three precursors at early translation before the synthesis of the viral protease suggests that a reticulocyte protease is responsible for their formation. We propose that this reticulocyte protease acts on nascent chains. Depending upon the conditions of the lysates, this enzyme catalyzes the formation of either protein A1 as the predominant product or a mixture of the three precursor proteins together.

Beyond the nascent chain stage, processing of A1, A, and B requires the intervention of the viral protease. For a closer examination of this process, we prepared a sample primarily containing protein A1. EMC RNA was translated in the lysate for 25 min in the presence of [^{35}S]-methionine. Sparsomycin was added to stop further translation. An unlabeled lysate mixture containing total EMC translation products and, thus, the viral protease, was then added. Figure 2 shows the polyacrylamide gel patterns of the samples taken at different time intervals. Lane a exhibits the zero-time sample which shows, as expected, protein A1 as the major protein along with a very small amount of protein A. Lane b shows that, 2.5 min after the mixing, the protein A1 band decreased to almost half its original intensity, whereas the intensity of the protein A band increased markedly. Protein B appeared as a light band at this time. In addition, two small proteins (marked by arrows) that migrated ahead of protein γ also increased. This change

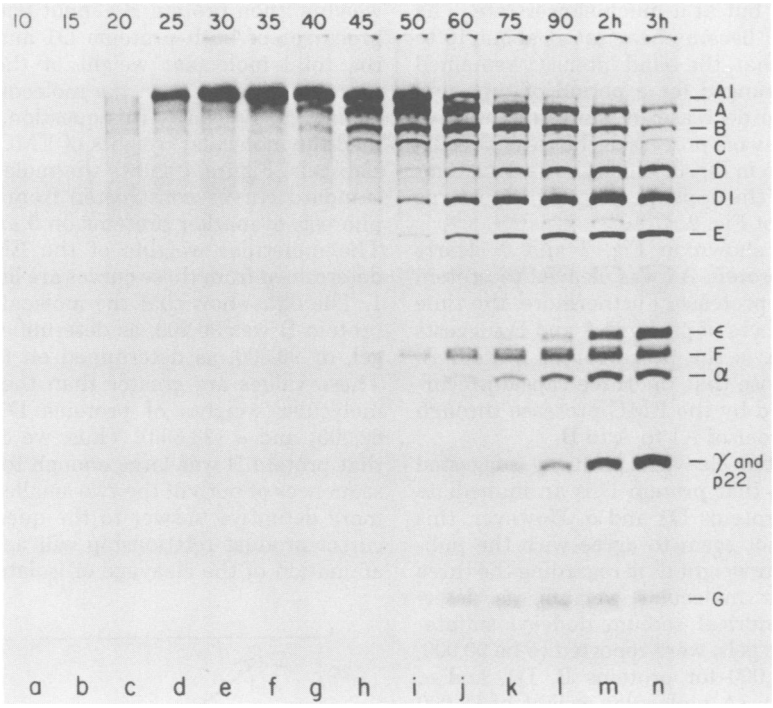

FIG. 1. *Formation of EMC proteins as a function of time. The conditions for protein synthesis and polyacrylamide gel electrophoresis were as previously described (4). The reaction mixture was 60 μl. Two-microliter samples were withdrawn at the indicated time intervals (in minutes unless otherwise indicated). Autoradiography was for 2 days.*

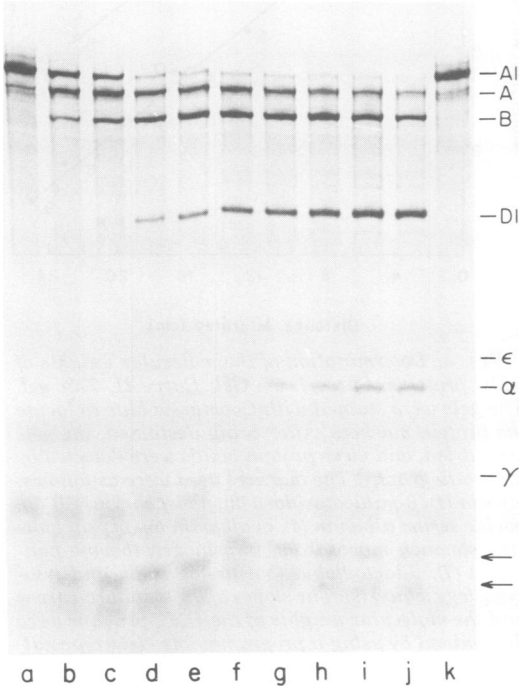

FIG. 2. *Cleavage of protein A1 by EMC protease. For preparation of labeled A1, translation was allowed in the presence of [^{35}S]methionine for 25 min. Sparsomycin was added to a final concentration of 0.2 mM. For preparation of EMC total translation products (as the source of EMC protease), a normal reaction mixture but without [^{35}S]methionine was prepared and incubated for 2 h. The two mixtures (60 μl each) were combined, and 2-μl samples were taken at 0 (a), 2.5 (b), 5 (c), 10 (d), 15 (e), 20 (f), 25 (g), 30 (h), 40 (i), and 50 (j) min. Lane k represents a control sample the substrate of which was mixed with a mock reaction mixture containing no EMC RNA and was incubated for 60 min. Electrophoresis conditions were the same as those described in the legend to Fig. 1, except that the acrylamide concentration was 7.5% instead of 10%. This figure is an autoradiogram of the gel.*

was not detected in a control sample (lane k) in which protein A1 was mixed with a mock lysate mixture containing no EMC protease. Lanes c and d show that protein A1 disappeared rapidly, and, by 15 min, the majority of protein A1 disappeared (lane e). Conversely, the protein A band increased rapidly. The band became most intense at 5 min (lane c). The protein B band

also increased, but at a much slower rate. The protein B band became heaviest at about 15 to 20 min. After that, the band intensity remained relatively unchanged for a period of time and then started to decrease. A more quantitative view of the cleavage process of the three precursors can be seen in Fig. 3, which shows densitometer tracings of the upper portion of the patterns of lanes a to f of Fig. 2.

The results shown in Fig. 2 and 3 clearly indicate that protein A1 was cleaved to protein A by the viral protease. Furthermore, the time of the appearances of proteins A and B suggests that protein A is the precursor of protein B. Thus, we propose that the three capsid precursors are cleaved by the EMC protease through a sequential order of A1 to A to B.

The sequential cleavage pathway suggested above predicts that protein B is an immediate precursor of proteins D1 and α. However, this scheme does not seem to agree with the published molecular weight data regarding the three proteins. Their molecular weights, as determined on cylindrical sodium dodecyl sulfate-polyacrylamide gels, were reported to be 90,000, 65,000, and 34,000 for proteins B, D1, and α, respectively (1). (A molecular weight of 32,000 was also reported for protein α based on guanidine hydrochloride-gel filtration results [2].). If these values represent the true molecular weights, then protein B cannot possibly be the precursor of both proteins D1 and α, because the total molecular weight of the latter two proteins is larger than the molecular weight of protein B. To clarify this question, we reexamined the molecular weights of EMC proteins on slab gels. Figure 4 shows the molecular weight standard curves constructed from the electrophoresis of marker proteins on 5 and 7.5% gels. The molecular weights of the EMC proteins determined from these curves are listed in Table 1. The data show that the molecular weight of protein B was 96,200, as determined on the 5% gel, or 96,300, as determined on the 7.5% gel. These values are greater than the sum of the molecular weights of proteins D1 (61,000 or 62,000) and α (33,600). Thus, we can conclude that protein B was large enough to contain the sequences of both of the two smaller proteins. A more definitive answer to the question of precursor-product relationship will necessitate examination of the cleavage of isolated protein B

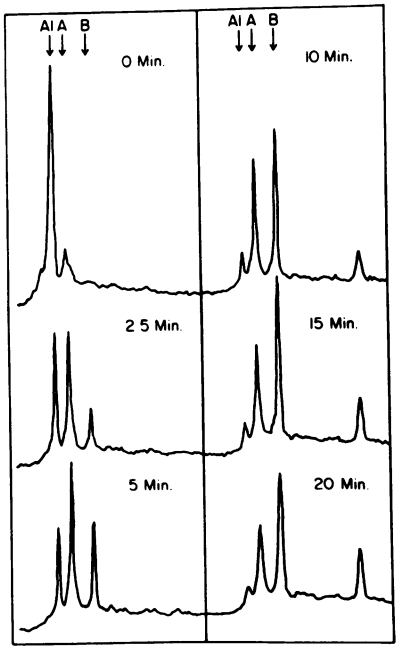

FIG. 3. *Densitometer tracings of the upper portion of the patterns of lanes a to f of Fig. 2.*

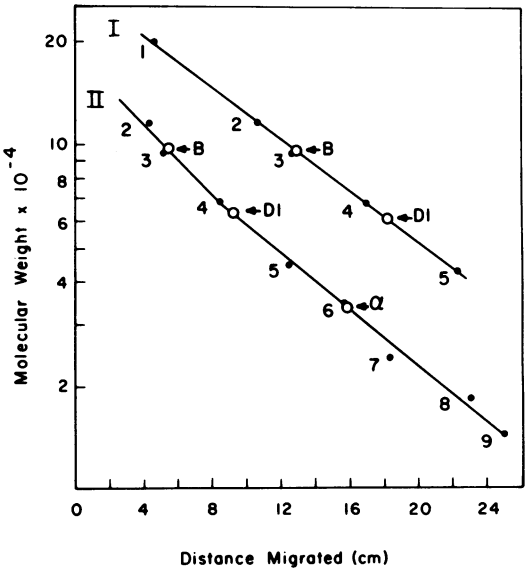

FIG. 4. *Determination of the molecular weights of EMC proteins. Curve I, 5% Gel; Curve II, 7.5% gel. The gels were stained with Coomassie blue to locate the protein markers. After being destained, the gels were dried, and virus protein bands were detected by autoradiography. The markers used were as follows: myosin (1), β-galacotosidase (2), phosphorylase B (3), bovine serum albumin (4), ovalbumin (5), pepsin (porcine stomach mucosa) (6), trypsinogen (bovine pancreas) (7), β-lactoglobin (bovine milk) (8), and lysozyme (egg white) (9). The slopes of the standard curves and the molecular weights of the EMC proteins were determined by using a programmable electronic calculator.*

TABLE 1. *Molecular weights of EMC proteins*

Protein	Mol wt determined on:	
	5% gel	7.5% gel
A1	121,300	117,300
A	109,500	107,200
B	96,200	96,300
C	86,800	88,500
D	72,500	74,300
D1	61,000	62,000
E		52,800
ϵ		40,700
F		38,600
α		33,600
γ		24,700
G		15,000

and analysis of the tryptic peptides of the three proteins.

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