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Characterization of Adenovirus-Associated Virus-Induced Polypeptides in KB Cells

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Electrophoretic analyses of KB cells coinfected with adenovirus-associated virus (AAV) type 2, a defective parvovirus, and adenovirus type 5 (as helper) have revealed the synthesis in vivo of at least five AAV-specific polypeptides. The three largest polypeptides, with molecular weights of 90,700, 71,600, and 60,000 comigrated in polyacrylamide gels with the three AAV structural polypeptides. The remaining two polypeptides had molecular weights of 24,900 and 15,800. The concentrations of the AAV-induced polypeptides relative to one another remained approximately constant during the infectious cycle, and the structural components were present in proportions similar to those found in purified virions. As determined by pulse-chase experiments, all polypeptides were generated at the level of protein synthesis and not by posttranslational proteolytic processing. Although inhibitors of proteolytic enzymes failed to influence the pattern of AAV-induced polypeptides, an amino acid analog, L-canavanine, blocked the appearances of both the major structural polypeptide (60,000 daltons) and the larger nonstructural polypeptide (24,900 daltons). Taken in conjunction with pulse-chase data, this result supports a model whereby the major virion polypeptide is produced by proteolytic cleavage of the nascent polypeptide chain.

Adenovirus-associated viruses (AAV) are members of the parvovirus group, a ubiquitous family of animal viruses that share numerous physical and biochemical features (14, 16). Whereas most parvoviruses multiply autonomously, the replication of AAV requires coinfection with a helper adenovirus (Ad). AAV particles have been shown to contain either a plus or a minus DNA strand with a molecular weight of 1.4×10^6 (16). The capsid proteins that enclose these strands consist of at least three polypeptides whose molecular weights were estimated previously to be 87,000, 73,000, and 62,000 (12, 19). These AAV structural polypeptides are apparently translated from a single, stable mRNA species with a molecular weight of about 9.0×10^5, which represents approximately 70% of genome sequences (7, 16).

At present, little is known concerning the actual means by which the AAV mRNA, whose theoretical coding capacity does not exceed 95,000 daltons, is translated into polypeptides totaling 222,000 daltons (19). This discrepancy and the antigenic homology between the 73,000- and 62,000-dalton polypeptides (11) predicts a scheme of synthesis that should provide for amino acid sequence overlap among the three structural polypeptides. The objective of this study, therefore, was to characterize AAV-induced polypeptides in vivo and to determine a probable mechanism for their synthesis.

MATERIALS AND METHODS

Materials. Amino acid analogs, L-ethionine, L-canavanine, L-azetidine-2-carboxylic acid, and DL-*p*-fluorophenylalanine, and protease inhibitors, L-1-tosylamide-2-phenylethyl-chloromethyl ketone, 1-chloro-3-tosylamido-7-amino-L-2-heptanone hydrochloride, and phenylmethyl sulfonylfluoride, were purchased from Sigma Chemical Co., St. Louis, Mo.

Cells and virus. KB cells (3×10^5 cells per ml) in suspension culture at 37°C were infected with Ad type 5 alone or with Ad5 and AAV type 2 simultaneously (Ad/AAV) at multiplicities of 10 50% tissue culture-infective doses per cell as described elsewhere (2). The Ad inoculum was a clarified cell lysate (8), whereas the AAV stock was a suspension of CsCl-purified virus heated at 56°C for 15 min to inactivate any contaminating Ad (16-18).

Isotopic labeling of intracellular proteins. Infected cultures (50 ml each) were centrifuged at 250 × *g* for 5 min at 20°C, and the cell pellets were suspended in modified Eagle medium containing either 10-fold-reduced or no L-methionine. After a preliminary incubation for 10 min to diminish the endogenous pool, radioactive labeling with L-[^{35}S]methionine (>300 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was carried out as described for individual experiments. If uniformly labeled polypeptides were desired, amino acid-free modified Eagle medium and *U*-^{14}C-labeled protein hydrolysate (54 mCi/matom of

carbon; Amersham/Searle, Arlington Heights, Ill.) were implemented as above. Labeling periods were terminated either by a chase with modified Eagle medium supplemented with 1,000 times the normal concentration of the cold amino acids or by centrifugation at 1,100 × *g* for 5 min at 4°C. After centrifugation, cell pellets were suspended in 150 μl of Tris-saline buffer (140 mM Tris–140 mM NaCl–5 mM KCl–0.7 mM Na_2HPO_4–5 mM dextrose, pH 7.4), sampled in duplicate to determine trichloroacetic acid-precipitable radioactivity, and stored in 50-μl portions above liquid nitrogen before polyacrylamide gel electrophoresis.

Radioactive AAV virion polypeptides. An Ad/AAV-infected culture (100 ml) in normal modified Eagle medium was incubated until 18 h postinfection (p.i.), and the cells were then concentrated 10-fold (i.e., to 3 × 10^6 cells per ml) in L-methionine or amino acid-free modified Eagle medium and were incubated with 100 μCi of either L-[^{35}S]methionine or *U*-^{14}C-labeled protein hydrolysate per ml for 30 min. The culture was next diluted to 100 ml with medium reduced 10-fold in the amino acid(s) corresponding to the radioactive label and maintained at 37°C until 22 h p.i., when the concentration of the deficient amino acid(s) was normalized. The infected cells were harvested at 30 h p.i., and virions were purified without trypsin treatment as described previously (17, 18).

Polyacrylamide gel electrophoresis. Total cell polypeptides were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis as described by Studier (23) and modified by Marsden et al. (15). Briefly, the slab gel was prepared in two stages. In the first step, a resolving gel composed of a linear gradient of 6 to 17% total acrylamide (5% in *N,N'*-methylenebisacrylamide) was cast between double-strength window glass (17 by 32.3 cm) sealed on three sides by TFE plastic film (tape no. 5490, 3.5-mil [ca. 0.87-mm] Teflon; Minnesota Mining & Manufacturing Co., St. Paul, Minn.). The second stage was performed just before electrophoresis, when a stacking gel (5% total acrylamide) was cast around a 10-tooth Teflon comb (Bio-Rad Laboratories, Richmond, Calif.). Samples, containing 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue, were boiled for 2 min before application to sample wells. Electrophoresis was carried out in a Bio-Rad support apparatus (model 221) at 20 mA of constant current for 17 h at 4°C.

Protein bands in the gel were stained with Coomassie brilliant blue R-250 for 1 h and destained in a methanol–acetic acid–water bath with gentle shaking (15). The destained gel was bonded to filter paper through a combination of heating and vacuum (Bio-Rad gel dryer, model 224) before autoradiography with Kodak X-Omatic royal purple film (Picker Corp., Falls Church, Va.). Autoradiograms were developed after a suitable period of exposure with conventional procedures, using Kodak developer and fixer.

Molecular weight estimates. The molecular weights of the following polypeptide standards were taken from Weber and Osborn (25) and Marsden et al. (15): bovine liver L-glutamate dehydrogenase (53,000), bovine hemiglobin (α-chain = 14,100, β-chain = 14,600), bovine liver catalase (60,000), bovine erythrocyte carbonic anhydrase (29,000), and *Escherichia coli* RNA polymerase (B' = 157,500, β = 150,000, σ = 90,000, and α = 40,000).

RESULTS

AAV-induced polypeptides in KB-3 cells. Although previous investigators have identified at least three AAV structural polypeptides (12, 19), the presence of additional AAV-specific polypeptides not incorporated into progeny virions has not been ruled out. Because adenovirus infection effectively inhibits cell-specific protein synthesis (13) and, thus, reduces the number of polypeptides labeled during a pulse with radioactive amino acids, sodium dodecyl sulfate-polyacrylamide gel electrophoresis should detect both structural and nonstructural AAV-specific polypeptides, which migrate with mobilities distinct from Ad-coded polypeptides and the residual products of host protein synthesis. To provide a more exact estimate of the number of AAV-induced polypeptides, one culture of KB cells was infected with Ad alone, and another was infected with Ad/AAV. At 20 h p.i., two 3-ml subcultures were prepared from each culture, and L-[^{35}S]methionine (20 μCi/ml) or [*U*-^{14}C]-labeled protein hydrolysate (50 μCi/ml) was added. After a 1-h labeling period, each subculture was harvested and processed for electrophoresis. Figure 1 (track b) reveals four bands (A, B, C, and D) radioactively labeled with L-[^{35}S]methionine that were specific to the Ad/AAV-infected culture. Furthermore, when *U*-^{14}C-labeled protein hydrolysate was used as the radiolabel of choice, a fifth band (E) was detected (Fig. 1, track c; see also Fig. 2, tracks f, g, and h, and Fig. 3, tracks a, c, e, and g), suggesting the presence of at least one polypeptide deficient in methionine residues. Of these five AAV-induced polypeptides, only A, B, and C were found in virions (Fig. 1, track e). Molecular weight estimates of these polypeptides are summarized in Table 1. The electrophoretic heterogeneity in the C polypeptide of purified marker virions (Fig. 1, track e) is probably due to the action of residual cell-specified proteases during repeated freezing and thawing.

Temporal appearance of AAV polypeptides. After a simultaneous coinfection with Ad, AAV-specific RNA and protein synthesis are initially detected by 10 and 14 h p.i., respectively (6, 11). Since AAV synthesizes only one stable mRNA species in vivo (7), it was important to determine whether the relative amounts of each virus-induced polypeptide remained constant during the course of infection. The temporal appearance and stoichiometric relationships of these polypeptides were, therefore, investigated

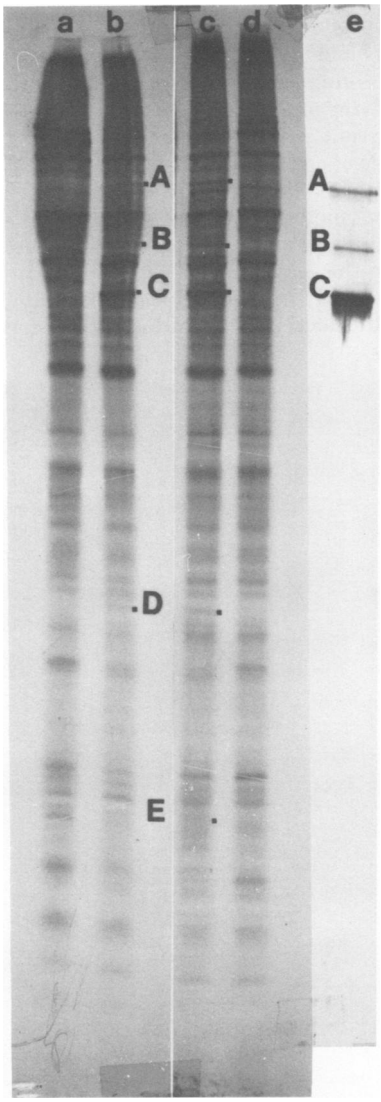

FIG. 1. *AAV-induced polypeptides in KB cells. An Ad-infected culture (tracks a and d) and an Ad/AAV-infected culture (tracks b and c) were pulsed at 20 h p.i. for 1 h with L-[^{35}S]methionine (20 μCi/ml; tracks a and b) or with U-^{14}C-labeled protein hydrolysate (50 μCi/ml; tracks c and d). AAV virion polypeptides (^{14}C-labeled) are shown in track e. Separation of polypeptides was achieved by electrophoresis in a 6 to 17% acrylamide gel.*

by pulsing 3-ml portions of Ad/AAV- or Ad-infected KB cells with 50 μCi of *U*-^{14}C-labeled protein hydrolysate per ml for 1.5 h at selected times after infection (Fig. 2). Virus structural polypeptides (A, B, and C) could be clearly demonstrated by 14 h p.i. (track d), whereas polypeptides D and E presumably were not present in sufficient quantities for detection until 18 h p.i. (track f). A temporal control of AAV structural polypeptide synthesis was not evident, as indicated by a close examination of Fig. 2. By visual inspection, the relative concentrations of virus-induced polypeptides remained approximately constant, and the structural polypeptides appeared to be present in the same proportions as seen with purified virions (compare with track a).

A summation of the individual molecular weights of the virus-induced polypeptides indicates that they exceed by 2.8-fold the theoretical coding capacity of the single virus message (Table 1). Although the synthesis of overlapping polypeptides from a single mRNA could, in theory, be accomplished by control of initiation or termination or by proteolytic processing of the nascent or completed polypeptides (or some combination of these processes), only the proteolytic mechanism has been observed in virus-infected cells (10, 21, 22). In an attempt to elucidate the mechanism of synthesis of the AAV-induced polypeptides, three kinds of experiments were carried out. In the first type of experimental approach, a short pulse of radioactive amino acids followed by a chase with excess non-radioactive amino acids was used to define whether production of AAV-induced polypeptides was directly coupled to protein synthesis (10, 22) or resulted from posttranslational cleavage of a primary product (1, 21). In the second and third types of experiments, proteolytic enzyme inhibitors and amino acid analogs were used to block the presumptive cleavage of a precursor polypeptide(s). With either class of compounds, interference with production of one or more of the AAV polypeptides could be interpreted as evidence for a proteolytic processing mechanism.

Stability of pulse-labeled AAV polypep-

TABLE 1. *Estimation of the molecular weights of AAV-2-induced polypeptides*

Gel component	Mol wt (×10^{-3})[a]	
A	90.7 ± 2.3[b]	(11)[c]
B	71.6 ± 1.6	(12)
C	60.0 ± 2.0	(12)
D	24.9 ± 1.7	(8)
E	15.8 ± 0.9	(7)

[a] A total of 263,000 daltons of polypeptides are induced by AAV-2 in KB cells.

[b] Maximum coding capacity of the AAV mRNA (molecular weight = 0.9 × 10^6) is 95,000 daltons of protein, as calculated from average nucleotide molecular weight (322), average amino acid molecular weight (110), and 3′ polyadenylate sequence of 200 (4, 5).

[c] Number of independent analyses.

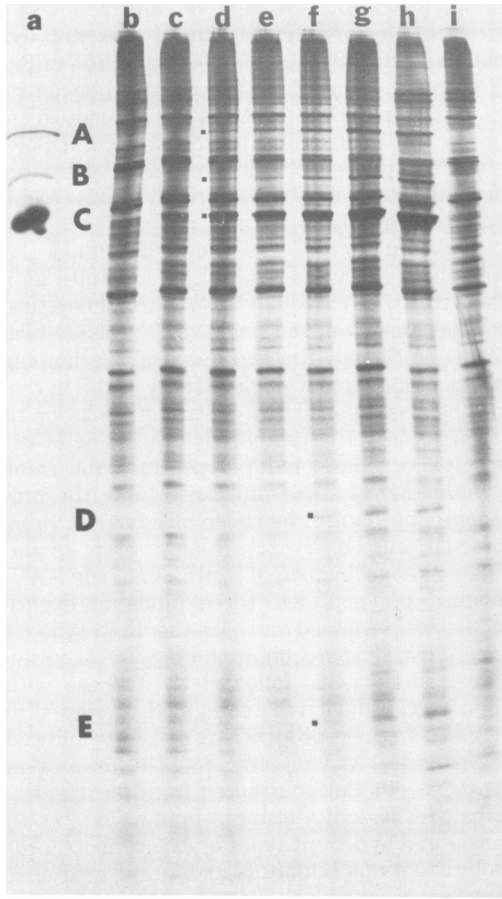

FIG. 2. *Appearance of AAV-induced polypeptides as a function of hours p.i. Ad/AAV-infected KB cells were pulsed with 50 μCi of U-^{14}C-labeled protein hydroylsate per ml for 1.5 h at 10 (track b), 12 (track c), 14 (track d), 16 (track e), 18 (track f), 20 (track g), and 25.5 (track h) h p.i. A control culture infected with Ad alone (track i) was similarly labeled at 16 h p.i. Virion polypeptides are shown in track a.*

tides. To detect rapid posttranslational processing, two 50-ml cultures of KB cells, one infected with Ad alone and the other infected with Ad/AAV, were harvested at 22 h p.i. and concentrated 50-fold in amino acid-free medium. After incubation for 7 min, *U*-^{14}C-labeled protein hydrolysate (1 mCi/ml) was added, and the incubation was continued for 1 min. Eagle chase medium (19 ml) was then added, and 4-ml samples were withdrawn for analysis by polyacrylamide gel electrophoresis at 0, 10, 20, and 30 min. The net incorporation of radioactive amino acids into trichloroacetic acid-precipitable radioactivity did not increase significantly over the entire chase period (R. M. L. Buller, unpublished data). In addition, no obvious precursor-product relationship was visible either in an Ad alone or in an Ad/AAV infection (Fig. 3). This finding argues strongly that, if occurring, proteolytic processing operates at the level of the nascent polypeptide as opposed to that of cleavage of a mature precursor polypeptide. The second faint AAV-specific band, which has an apparent molecular weight slightly smaller than that of polypeptide B, may represent heterogeneity in the processing of A to B.

Effect of inhibitors of proteolysis on AAV-induced polypeptides. Others have suc-

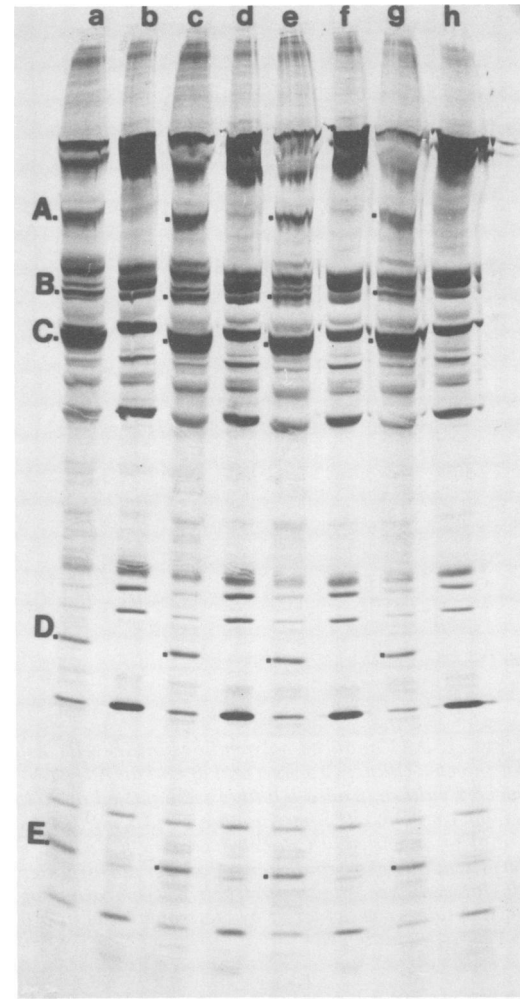

FIG. 3. *Stability of pulse-labeled AAV-induced polypeptides in KB cells. KB cells infected with either Ad (tracks b, d, f, and h) or Ad/AAV (tracks a, c, e, and g) were pulsed at 20 h p.i. for 1 min with U-^{14}C-labeled protein hydrolysate (1 mCi/ml) and chased with 1,000 times the normal concentration of amino acids for 0 (tracks a and b), 10 (tracks c and d), 20 (tracks e and f), and 30 (tracks g and h) min.*

cessfully used protease inhibitors to demonstrate the proteolytic processing of nascent polio polypeptides by chymotrypsin-like enzymes (24). Similar experiments with AAV-infected cells were, therefore, carried out. A series of Ad/AAV- and Ad-infected cultures (6 ml each) were treated at 19 h p.i. with 0.5% dimethyl sulfoxide and 1-chloro-3-tosylamido-7-amino-L-2 heptanone hydrochloride (10^{-4} M), L-1-tosylamide-2-phenylethyl-chloromethyl ketone (10^{-5} M), phenylmethyl sulfonylfluoride (10^{-4} M), or no inhibitor. After a 10-min incubation, L-[^{35}S]-methionine (20 μCi/ml) was added, and each culture was maintained at 37°C for an additional 2 h before harvesting. In comparison with control values, the respective effects of these protease inhibitors on overall protein synthesis (as measured by L-[^{35}S]methionine incorporation into an acid-insoluble product) were 110% (1-chloro-3-tosylamido-7-amino-L-2-heptanone hydrochloride), 52% (L-1-tosylamide-2-phenylethyl-chloromethyl ketone), and 129% (phenylmethyl sulfonylfluoride). The electropherogram (Fig. 4) indicates no obvious reduction in polypeptide C synthesis, at least at the inhibitor concentrations used. The diminished intensities of the A and B bands in the control and test samples resulted from a shorter-than-usual period of autoradiography.

Effect of amino acid analogs on AAV-induced polypeptides. Since a class of cell-associated proteases may exist that is unaffected by the protease inhibitors used, several amino acid analogs were tested in an attempt to detect a possible precursor polypeptide in association with a concomitant reduction of AAV-induced structural and nonstructural polypeptides. Preliminary studies with DL-*p*-fluorophenylalanine (20 mM), L-canavanine (10 mM), L-azetidine-2-carboxylic acid (10 mM), and L-ethionine (10 mM) (alone and combined) revealed that only L-canavanine affected the generation of AAV-induced polypeptides. Although the concentration of L-canavanine used (10 mM) abolished the appearance of polypeptides C and D, a precursor with a molecular weight greater than that of polypeptide A (90,700) was not seen. Additionally, polypeptides A and B continued to be synthesized in the presence of L-canavanine. In a further attempt to block the synthesis of the larger structural polypeptides (so that any precursor larger than polypeptide A might be detected), the effects of a range of L-canavanine concentrations were examined. At 20 h p.i., a series of 3-ml subcultures of Ad/AAV- or Ad-infected KB cells were incubated in the presence of either no analog or 5, 10, 20, or 40 mM L-canavanine. The radioactive amino acid, L-[^{35}S]methionine (50 μCi/ml), was then added for a 1-h labeling period, and cell pellets were collected and analyzed by polyacrylamide gel electrophoresis. Identical AAV-induced polypeptide patterns (Fig. 5, tracks e through h) were obtained irrespective of the concentration of L-canavanine used. This analysis was again characterized by the absence of polypeptides C and D as well as the absence of any new poly-

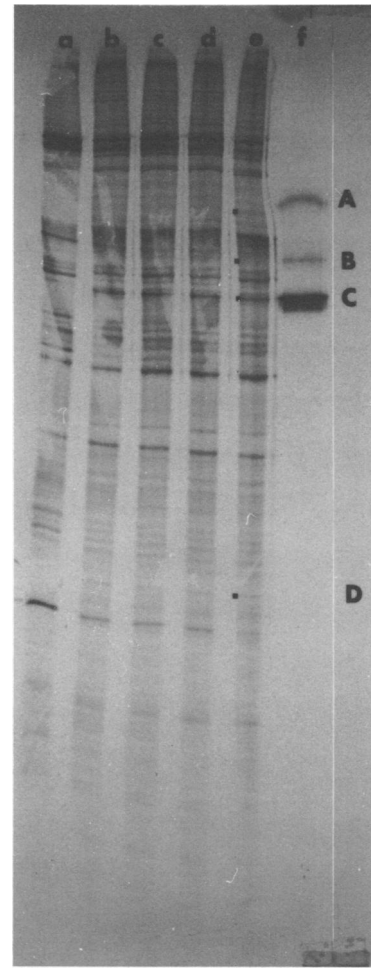

FIG. 4. *Effect of protease inhibitors on the synthesis of the major structural polypeptide. Ad/AAV-infected cells were incubated in the presence of 0.5% dimethyl sulfoxide at 19 h p.i. for 10 min with no inhibitor (track b), 10^{-4} M 1-chloro-3-tosylamido-7-amino-L-2-heptanone hydrochloride (track c), 10^{-5} L-1-tosylamide-2-phenylethyl-chloromethyl ketone (track d), and 10^{-4} M phenylmethyl sulfonylfluoride (track e). Each incubation was continued for an additional 2 h in the presence of L-[^{35}S]methionine (20 μCi/ml). An untreated Ad-infected culture was similarly labeled with L-[^{35}S]methionine (track a). Virion polypeptides, labeled with L-[^{35}S]methionine, are analyzed in track f.*

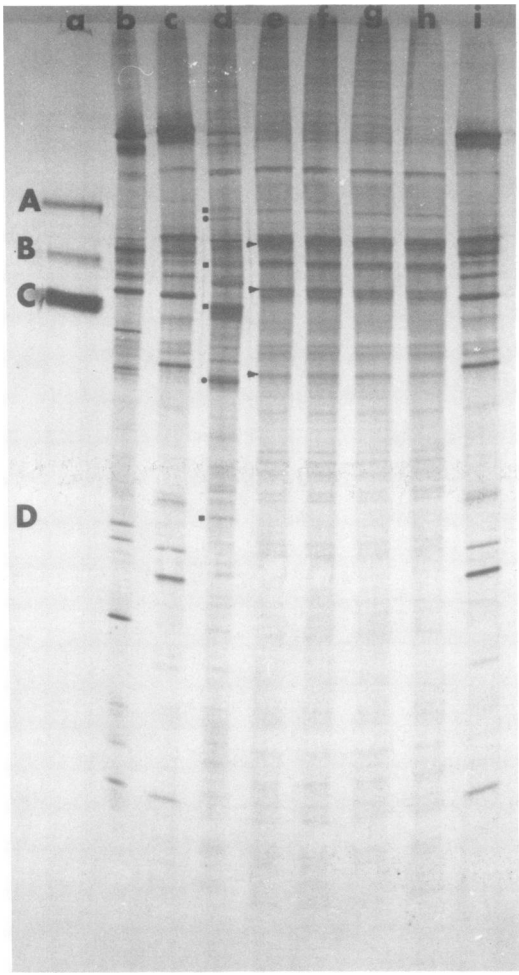

FIG. 5. *Effect of* L-*canavanine on the synthesis of AAV-induced polypeptides. A series of Ad-infected (tracks b, c, and i) or Ad/AAV-infected (tracks d through h) KB cell cultures were labeled at 20 h p.i. with* L-[^{35}S]*methionine (50 μCi/ml) for 1 h in the presence of either no inhibitor (tracks b and d) or the following concentrations of* L-*canavanine: 5 mM (tracks c and e), 10 mM (track f), 20 mM (track g), and 40 mM (tracks h and i). Virion polypeptides, labeled with* L-[^{35}S]*methionine, are analyzed in track a. Symbols: (▶) Polypeptide specific to* L-*canavanine treatment; (●) inconsistently detected, Ad/AAV-specific polypeptide.*

peptide that could be considered a precursor of polypeptide A. However, three novel polypeptides specific to L-canavanine treatment of Ad/AAV-infected cells were produced (track e, arrows). The two larger polypeptides, 80,000 and 65,100 daltons, were consistently observed during independent analyses, whereas detection of the smallest polypeptide was more variable. Another effect of L-canavanine treatment can be seen in the apparent increase in material that comigrated with authentic B polypeptide (tracks e through h). It should also be noted that, although DL-*p*-fluorophenylalanine did not appear to affect the synthesis of A, B, C, and D polypeptides, small amounts of a polypeptide that comigrated with the L-canavanine-induced 65,100-dalton polypeptide were detected (Buller, unpublished data). A subtle variability in the synthesis of certain AAV-induced polypeptides is demonstrated by the presence of two polypeptides (track d, circles) in the control Ad/AAV-infected cells. Although specific to the Ad/AAV infections, these polypeptides (85,700 and 45,600 daltons) were not detected consistently.

DISCUSSION

Examination of Ad/AAV-infected KB cells by sodium dodecyl sulfate-polyacrylamide gel electrophoresis has revealed at least five polypeptides specific to AAV-2 infection (Fig. 1). The detection of these polypeptides in African green monkey kidney cells infected with AAV-2, Ad5, and simian virus 40 further substantiates their AAV-specified origin (Buller, unpublished data). In addition, recent in vitro studies with purified AAV-2 mRNA have demonstrated the virus specificity of all five AAV polypeptides induced in vivo (Buller, unpublished data). Of these five polypeptides, the three largest (A, B, and C) comigrated with the structural polypeptides of highly purified AAV virions. The structural polypeptides could be identified with certainty by 14 h p.i. (Fig. 2), approximately 6.5 and 4 h, respectively, after the onset of detectable DNA and RNA synthesis (6) and 3 h before the appearance of progeny virus (16). The relative proportions of the three structural polypeptides remained fairly constant during the virus growth cycle and seemed to be present in the same proportions as found in purified virions.

Using short pulses of radioactivity, pulse-chase experiments strongly suggest that all of the virus polypeptides detected arose during nascent synthesis and not by posttranslational processing (Fig. 3). This latter mechanism has been observed in cells infected with Sindbis virus (21), Newcastle disease virus (20), and Ad (1).

Excluding posttranslational cleavage, there are at least three basic mechanisms by which the lower-molecular-weight capsid polypeptides might be generated during protein synthesis: there may be (i) more than one functional initiation site or (ii) more than one termination site, or (iii) proteolytic sizing might occur while synthesis of the polypeptide chain is in progress. At present, there is no experimental evidence in mammalian systems for more than one active initiation site in each mRNA. Although studies on the initiation of polypeptide synthesis with

tobacco mosaic (9) and Sindbis (3, 22) viruses have shown that virion RNA (which can act as mRNA in vivo) contains at least two sites for the initiation of protein synthesis, only one major site appears to be functional in vitro (3, 9, 22) and, presumably, in vivo. If there is some constraint in eucaryotic cells that prohibits the expression of a second initiation site in mammalian mRNA, then proteolytic cleavage of the growing polypeptide could be the sole mechanism whereby more than one polypeptide could be synthesized from a unique initiation site (3, 10). Considering the nonstructural polypeptides, D and E, one can devise a scheme whereby one nascent proteolytic cleavage produces the B (71,600 daltons) and E (15,800 daltons) polypeptides or the C (60,000 daltons) and D (24,900 daltons) polypeptides (Fig. 6, model 1). In addition, AAV-induced polypeptides with molecular weights of 78,000 and 66,000 have occasionally been detected and could represent intermediary cleavage products of the B and C polypeptides, respectively (Buller, unpublished data; Fig. 6, model 2). Consistent with this latter possibility is the fact that during L-canavanine treatment (which blocks the synthesis of polypeptides C and D) of Ad/AAV-infected cells, two polypeptides, 80,000 and 65,100 daltons (Fig. 5, tracks e through h), were synthesized. These AAV-specific L-canavanine-associated polypeptides could represent the buildup of normal cleavage intermediates that are only rarely seen in the course of an untreated infection (Fig. 6, model 2). Alternatively, they might represent the products of aberrant processing events resulting from the exposure of previously cryptic cleavage sites. Although the disappearance of C and D polypeptides during L-canavanine treatment lends support to a model whereby both polypeptides arise after a single cleavage of the A polypeptide (Fig. 6, models 1 and 2), polypeptide C could also be generated by a further cleavage of the B polypeptide. This alternate mode of producing the C polypeptide is suggested by the apparent buildup of B-like polypeptide during L-canavanine treatment. In addition, the production of C polypeptide both directly from A and indirectly from B would explain why the apparent molar ratios at C to D are not equal. Because there is no strong support for a single means by which polypeptide C is generated, more than one cleavage scheme could be involved.

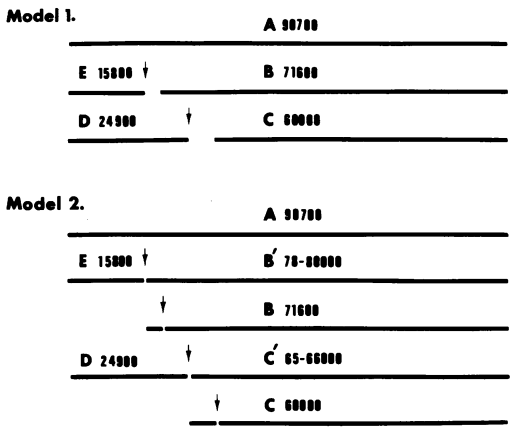

FIG. 6. *Schematic representation of two possible models for the generation of AAV-induced polypeptides during protein synthesis in KB cells. Model 1 requires a minimum of two cleavage sites to generate all of the major AAV-induced polypeptides. The gap adjacent to each cleavage site denotes an apparent absence of certain sequences originally present in the 90,700-dalton polypeptide. Model 2, on the other hand, includes precursors (B' and C') to both the B and the C polypeptides to explain the effect of L-canavanine on protein synthesis. It is not known which end contains the amino terminus.*

The fact that AAV appears to synthesize its polypeptide complement by means of a proteolytic cleavage mechanism that operates at the level of nascent polypeptide synthesis is not unique. In the case of poliovirus, proteolytic processing as a means to generate more than one polypeptide from a single mRNA also has been found to occur at the level of protein synthesis (10). However, the initial products of nascent processing appear to be sequence-unique polypeptides, whereas all AAV-specified polypeptides are, presumably, sequence subunits of the 90,200-dalton polypeptide. This latter feature is distinctive, and it is likely that most, if not all, parvoviruses derive their polypeptide complement in a similar fashion (16). In view of the finding that the relative proportions of newly synthesized AAV structural polypeptides are comparable to the proportions of these polypeptides found in purified virions, we also conclude that proteolytic processing is closely mediated by some regulatory mechanism. One possibility for the utility of such a specific control over polypeptide cleavages is that virion morphogenesis might be more effectively promoted if correct proportions of structural polypeptides are present at the site of assembly.

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