JOURNAL OF VIROLOGY, Mar. 1980, p. 954-961
0022-538X/80/03-0954/08$02.00/0

Vol. 33, No. 3

Effect of Canavanine on Murine Retrovirus Polypeptide Formation

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Canavanine is an arginine analog which is widely used to inhibit proteolytic processing of viral polyproteins. Certain results obtained with canavanine have suggested that it may have other effects. Therefore, we examined the effects of canavanine on the cell-free synthesis of murine retrovirus proteins. It was found that the electrophoretic mobility of the major *gag*-related cell-free product of both Rauscher murine leukemia virus (R-MuLV) and Moloney murine sarcoma virus 124 (Mo-MuSV-124) RNA was dependent on the concentration of canavanine used during translation. As the canavanine concentration was increased up to 4 mM, the apparent size of the major *gag*-related polypeptide also increased from 65,000 (R-MuLV RNA) or 63,000 (Mo-MuSV-124 RNA) to approximately 80,000 daltons. Additional increases in the canavanine concentration up to 12 mM did not increase the size of the *gag* gene product beyond 80,000 daltons. This change in electrophoretic mobility appeared to be due to a substitution of canavanine for arginine residues in the polypeptides, not to a change in their actual size. If amber suppressor tRNA and canavanine were used together during translation of Mo-MuSV-124 RNA and Mo-MuLV RNA, the results were also in agreement with this proposal. Translation experiments done with ovalbumin mRNA and mengovirus 35S RNA indicated that canavanine incorporation caused a shift in the electrophoretic mobility of ovalbumin from 43,000 to 45,000 daltons and caused the appearance of two slightly larger polypeptides in the 155,000- and 115,000-dalton regions of the mengovirus RNA cell-free product.

In Rauscher murine leukemia virus (R-MuLV)-infected cells and in the translation product of R-MuLV RNA, the predominant *gag*-related precursor polypeptide is $Pr65^{gag}$ (1, 9, 15, 24, 26). In infected cells, $Pr65^{gag}$ is cleaved to yield the viral core proteins (1, 2, 24, 26). However, if canavanine, an arginine analog, is substituted for arginine during pulse-chase experiments in infected cells or during cell-free translation of R-MuLV RNA, an 80,000-dalton (80K) *gag* gene product, originally designated $Pr80^{gag}$, accumulates, whereas $Pr65^{gag}$ either is not detected or is greatly diminished (1, 2, 9, 10, 16, 17, 24, 26). This result suggested that the 80K polypeptide was the primary *gag* precursor. This suggestion was strengthened by the observation that in R-MuLV-infected cells in the absence of canavanine, there is an 80K *gag*-related polypeptide which has a half-life of about 15 min (9) and a tryptic map almost identical to that of $Pr65^{gag}$ (1). In whole cells, in addition to its effects on the core protein precursors, canavanine blocks the formation of the mature viral proteins and the release of virus (9, 10).

Recently, we have been led to doubt the interpretation that the 80K *gag*-related polypeptide which accumulates in the presence of canavanine is the primary *gag* gene product. It was reported recently (6, 23) and has since been confirmed by us (V. Ng and R. Arlinghaus, unpublished data) that the 80K gag-related polypeptide observed in infected cells in the absence of canavanine is glycosylated and for this reason is now designated $gPr80^{gag}$, thus making it an unlikely candidate for a core protein precursor. In addition, intracellular $gPr80^{gag}$ lacks a p30-characteristic tryptic peptide found in both $Pr65^{gag}$ (11) and $Pr75^{gag}$, another intermediate *gag* precursor (E. C. Murphy and M. Nash, unpublished data), and contains a mannose-labeled tryptic peptide not found in $Pr65^{gag}$ (J. Kopchick and R. Arlinghaus, unpublished data). Hence, it seems probable that authentic $gPr80^{gag}$ and the 80K *gag*-related polypeptide which accumulates in the presence of canavanine are not the same polypeptide.

In this report, evidence will be presented which strongly suggests that canavanine incorporation during cell-free translation of R-MuLV $Pr65^{gag}$ and Moloney murine leukemia virus (Mo-MuLV) $Pr63^{gag}$ can cause a shift in electrophoretic mobility, the extent of which is depend-

ent on the concentration of canavanine used. At its maximum, canavanine appears to be able to change the electrophoretic mobility of both $Pr63^{gag}$ and $Pr65^{gag}$ such that they migrate with an apparent molecular weight of about 80,000. This change in electrophoretic mobility is probably not due to any change in the size of $Pr63^{gag}$ or $Pr65^{gag}$, but is instead due to a substitution of canavanine for arginine in these polypeptides. Canavanine incorporation exerts a similar but less dramatic effect on the cell-free product of mengovirus 35S RNA and ovalbumin RNA. Results with canavanine and amber suppressor tRNA used in combination are also in agreement with this proposal.

MATERIALS AND METHODS

Cells and viruses. For the production of R-MuLV, infected NIH Swiss mouse cells (JLS-V16) were grown in a modified Eagle medium in 2-qt (about 1.9-liter) roller bottles as described previously (25). Both clone 1 Mo-MuLV-infected cells (a generous gift of Karen Beemon) and Moloney murine sarcoma virus (Mo-MuSV-124)-infected cells (a generous gift of Peter Duesberg and also Judy Ball) were grown in roller culture in McCoy 5A medium containing 15% fetal calf serum. The Mo-MuSV-124-infected cells (clone G8-124) produce sarcoma virus against a low background of helper leukemia virus (3). Virus was harvested from the culture fluids by polyethylene glycol precipitation and purified by isopycnic banding in a 15 to 60% linear sucrose gradient (25).

Uninfected JLS-V16 cells, grown in a modified Eagle medium (25), were used as a source of the S-10 extracts used in the cell-free protein synthesis assays.

Isolation of viral nucleic acids. Viral RNA was isolated from the purified virus by sodium dodecyl sulfate (SDS)-phenol/chloroform extraction as described previously (15, 17), except that the virus was exposed to 100 μg of proteinase K per ml in 10 mM Tris (pH 7.5)-100 mM LiCl-1 mM EDTA-0.5% SDS for 15 min at 37°C prior to the organic extractions. Genome-size (70S) RNA was isolated from the R-MuLV and Mo-MuLV nucleic acids by centrifugation in a 5 to 25% sucrose gradient containing 10 mM Na(OAc) (pH 5.1)-100 mM LiCl-1 mM EDTA-0.1% SDS for 18 h at 15,000 rpm at 40°C. To obtain genome-size RNA from Mo-MuSV-124, the centrifugation speed was increased to 18,000 rpm. Subunit-size (35S for R-MuLV and Mo-MuLV; 30S for Mo-MuSV-124) RNA was isolated by sucrose gradient centrifugation as described previously (15, 17).

Whole chick oviduct polyadenylic acid-containing RNA was a generous gift of Savio Woo (Baylor College of Medicine). Mengovirus 35S RNA was a generous gift of George Thornton (Abilene Christian University).

Cell-free protein synthesis. The cell-free protein synthesis system and its preparation have been previously described (17). In essence, it is micrococcal-nuclease-treated (20) extract from uninfected JLS-V16 cells supplemented with a 0.5 M KCl wash of rabbit reticulocyte polyribosomes. The translation assays contain, in 25 μl: 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.6), 90 mM KCl, 1 mM $Mg(OAc)_2$, 150 μM spermidine, 8 mM 2-mercaptoethanol, 1 mM ATP, 150 μM GTP, 600 μM CTP, 10 mM creatine phosphate, 16 μg of creatine kinase per ml, 400 to 800 μCi of [^{35}S]methionine per ml (~1,200 Ci/mmol) or 500 μCi of [^{3}H]tyrosine per ml (55 Ci/mmol), 100 μM each of unlabeled amino acids, 5.25 μl of nuclease-treated cell extract, and 0.10 to 0.125 A_{280} (absorbance at 280 nm) units of rabbit reticulocyte ribosome high-salt-wash factors (7). Viral RNA was usually added at 40 μg/ml, and protein synthesis was allowed to proceed for 150 min at 30°C. Incorporation of radioactivity into protein was measured by hot trichloroacetic acid precipitation (17). When necessary, crude yeast suppressor-minus tRNA, amber suppressor tRNA, or ochre suppressor tRNA (a generous gift of Ray Gesteland) was added to a concentration of 20 to 40 μg/ml in the assay.

Gel electrophoresis. Polyacrylamide gel electrophoresis in 6 to 12% linear gradient gel slabs (or 11.25% slabs) in 0.1% SDS was performed, using the buffer system described by Laemmli (12). Fluorography was performed on the dried gels according to Bonner and Laskey (4). A linear response to radioactivity was obtained by preflashing the X-ray film (13). In some experiments, a New England Nuclear product (EN^{3}HANCE) was used instead of dimethyl sulfoxide-2,5-diphenyloxazole (DMSO-PPO) to impregnant the gels with fluor.

Quantitative estimates of the amount of radioactivity in individual polypeptides were obtained by excising gel slices containing these polypeptides from the gel slabs. Each slice was hydrated with 100 μl of water and incubated in 5 ml of NCS counting fluor (10 ml of NCS solubilizer:4.4 ml of Scinti-prep:85.6 ml of toluene) at 37°C for 16 h. After chilling, the radioactivity in each slice was determined and divided by the apparent molecular weight of the polypeptide to give an estimate of approximate molar yield.

RESULTS

Dependence of the electrophoretic mobility of major *gag*-related cell-free product on the concentration of canavanine. R-MuLV 35S RNA was translated in the presence of either 0.8, 2, 4, 8, or 12 mM canavanine. The results of this experiment (Fig. 1a) show that in the absence of canavanine, the major *gag*-related cell-free product had an apparent molecular weight of 65,000 (Fig. 1a, lane C). In 0.8 mM canavanine, no $Pr65^{gag}$ was seen; instead, a doublet of 70,000 to 73,000 molecular weight was observed (Fig. 1a, lane D). In 2 mM canavanine, the major *gag*-related polypeptide had an apparent molecular weight of 75,000 (Fig. 1a, lane E), and in 4, 8, and 12 mM canavanine (Fig. 1a, lanes F, G, and H, respectively), the molecular weight of this polypeptide doublet had shifted

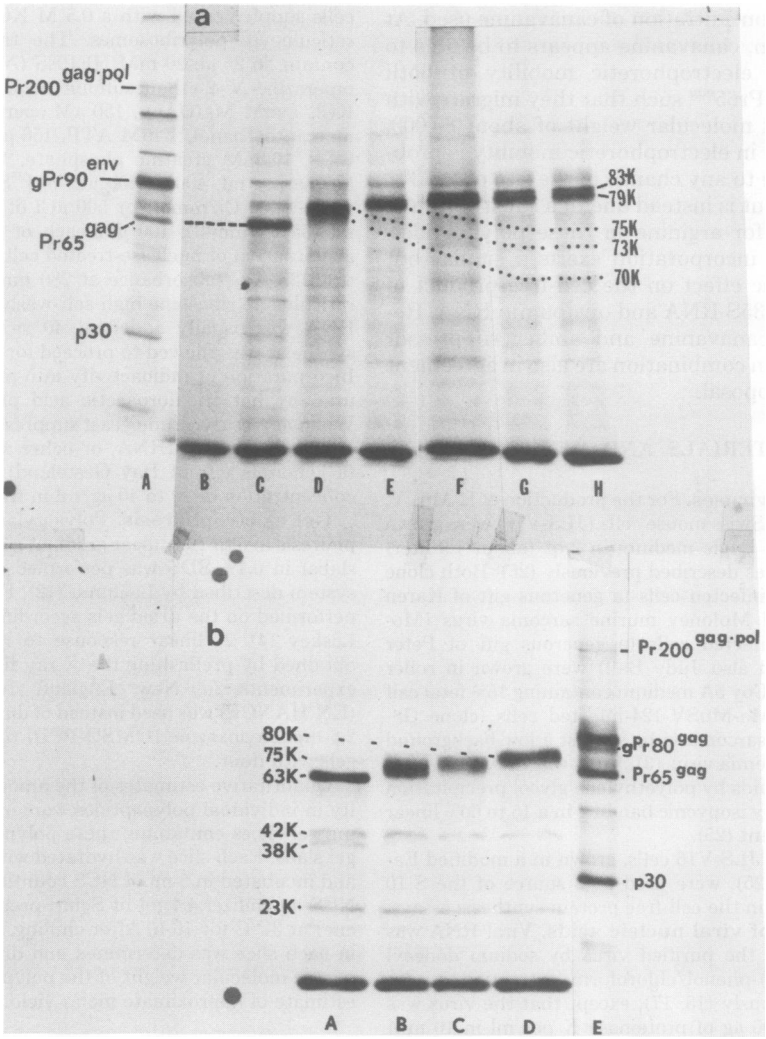

FIG. 1. *Translation of retrovirus RNA in the presence of canavanine. (a) Translation of R-MuLV 35S RNA. R-MuLV 35S RNA was translated in the presence of varying concentrations of canavanine, and the product was analyzed on a 6 to 12% polyacrylamide gel slab. (Lane A) An R-MuLV protein standard; (lane B) no RNA, no canavanine; (lane C) R-MuLV RNA, no canavanine; (lane D) R-MuLV RNA, 0.8 mM canavanine; (lane E) R-MuLV RNA, 2 mM canavanine; (lane F) R-MuLV RNA, 4 mM canavanine; (lane G) R-MuLV RNA, 8 mM canavanine; (lane H) R-MuLV RNA, 12 mM canavanine. (b) Translation of Mo-MuSV-124 RNA. Mo-MuSV-124 50-70S RNA was translated in the presence of varying concentrations of canavanine, and the product was analyzed on a 6 to 12% polyacrylamide gel slab. (Lane A) No canavanine; (lane B) 0.8 mM canavanine; (lane C) 1.6 mM canavanine; and (lane D) 4 mM canavanine; (lane E) the R-MuLV protein standard.*

to 79,000 to 83,000. We emphasize here that the shifts observed are shifts in electrophoretic mobility and do not necessarily imply an actual change in molecular weight. In many experiments, the polypeptide doublets were not resolved; instead, the product formed in the presence of canavanine migrated as a wide, somewhat diffuse band of approximately 80,000 molecular weight. We have previously shown that the 80K polypeptide (79–83K doublet) translated from R-MuLV 35S RNA in the presence of canavanine is *gag* related immunologically and possesses a methionine tryptic map indistinguishable from that of $Pr65^{gag}$ (15). Polypeptides smaller than $Pr65^{gag}$ appeared to be mostly unaffected by canavanine. This result could possibly be explained by their relatively low arginine content. These polypeptides are mostly prema-

turely terminated $Pr65^{gag}$ molecules containing p15, p12, and a portion of p30, and lacking p10 (16). Amino acid analyses have shown that p15 contains approximately 4 arginine residues, p12 contains 6 arginines, p30 contains 30 arginines, and p10 contains 14 arginines (5). Thus, the bulk of the arginine residues in $Pr65^{gag}$ has probably been lost in these premature termination fragments.

A similar dose dependence of the size of the major *gag*-related cell-free product of Mo-MuSV-124 50–70S RNA was also observed. The major Mo-MuSV-124 cell-free product translated in the absence of canavanine had an apparent molecular weight of 63,000 designated $P63^{gag}$ (Fig. 1b, lane A). However, in 0.8, 1.6, and 4 mM canavanine, the electrophoretic mobility of this product had shifted such that the polypeptide size appeared to be 67,000 to 70,000, 70,000 to 75,000, and 78,000 daltons, respectively (Fig. 1b, lanes, B–D).

Although other interpretations are possible, the dose dependence of the canavanine results suggest that a canavanine incorporation might be causing a shift in the electrophoretic mobility of $Pr65^{gag}$ and $P63^{gag}$ rather than having an effect on a processing step.

Effect of canavanine on amber suppression of polypeptide termination. It has recently been reported that translational control of the murine retrovirus joint *gag-pol* precursor may be mediated by suppressors of a UAG (amber) codon (21). We ourselves have found that Mo-MuLV $Pr200^{gag\text{-}pol}$ synthesis can be enhanced 20- to 40-fold at the apparent expense of $Pr65^{gag}$ by amber suppressor tRNA. In addition, we have observed that amber suppression of Mo-MuSV-124 $P63^{gag}$ termination leads to the formation of $Pr67^{gag}$ (Murphy and Arlinghaus, submitted for publication). In view of the apparent presence of a suppressible UAG codon at the end of the mRNA coding for Mo-MuSV $P63^{gag}$, it follows that the synthesis of a 78K *gag*-related polypeptide in the presence of canavanine must (Fig. 1b) involve the read-through of this proposed UAG codon if the 78K polypeptide is indeed a precursor to $P63^{gag}$ and not an artifact. However, if the 78K polypeptide synthesized in the presence of canavanine is in reality $P63^{gag}$ whose electrophoretic mobility has been shifted, then amber suppressor tRNA might be expected to allow the synthesis of a polypeptide about 4,000 daltons larger (analogous to $Pr67^{gag}$). The results of this type of an experiment are shown in Fig. 2a. In the absence of either canavanine or amber suppressor, the major Mo-MuSV-124 *gag*-related polypeptide had an apparent molecular weight of 63,000 (Fig. 2a, lane B). Amber suppression allowed the synthesis of a 67K polypeptide ($Pr67^{gag}$) at the expense of $P63^{gag}$ (Fig. 2a, lane C; Table 1). Translation of Mo-MuSV-124 RNA in 4 mM canavanine yielded a 78K polypeptide, but no $P63^{gag}$ or $Pr67^{gag}$ was observed (Fig. 2a, lane D). In the presence of both 4 mM canavanine and amber suppressor tRNA, an 84K polypeptide appeared in the Mo-MuSV-124 cell-free product in addition to the 78K polypeptide (Fig. 2a, lane E). The polypeptide in Fig. 2a, lane E, just slightly larger than 84,000 daltons is probably the translation artifact also seen in Fig. 2a, lanes B, C, and D. Quantitation of the results of this experiment showed that in the presence of amber suppressor tRNA, about 1 mol of $Pr67^{gag}$ was synthesized for every mole of $P63^{gag}$ lost. In the presence of canavanine and amber suppressor tRNA, for every mole of 78K polypeptide lost, approximately 1 mol of 84K polypeptide was synthesized. Although other explanations for the results of this experiment are conceivable, it seems most likely that the 78K polypeptide observed in canavanine is in reality $P63^{gag}$, and that the 84K polypeptide observed in the presence of both canavanine and amber suppressor tRNA is actually $Pr67^{gag}$.

Translation of Mo MuLV 35S RNA in the presence of both canavanine and amber suppressor also yielded results which are hard to explain unless it is presumed that canavanine incorporation changes the electrophoretic mobility of the gag precursor, $Pr63^{gag}$. In the presence of amber suppressor tRNA, $Pr200^{gag\text{-}pol}$ synthesis was increased greatly at the apparent expense of $Pr63^{gag}$ (compare lanes A and B in Fig. 2b; refer to Table 2). Cell-free synthesis in the presence of 4 mM canavanine resulted in the replacement of $Pr63^{gag}$ (Fig. 2b, lane A) with an 80K polypeptide (Fig. 2b, lane C). Amber suppressor tRNA appeared to mediate the efficient suppression of the 80K polypeptide synthesis to form $Pr200^{gag\text{-}pol}$ (Fig. 2b, lane D). Note that inclusion of 4 mM canavanine in the translation assay (Fig. 2b, lanes C and D), regardless of whether amber suppressor tRNA was also added, increased by 10,000 to 20,000 daltons the apparent molecular size of $Pr200^{gag\text{-}pol}$ and polypeptides a through e in the 80-to-200K region of the gel (compare Fig. 2b, lanes A and C, as well as lanes B and D). It was not clear whether the mobility of polypeptides f and g (Fig. 2b, lane D) was affected by canavanine. This result strengthens the argument that canavanine incorporation may be causing an artifactual increase in the electrophoretic mobility of polypeptides. Table 2 contains quantitative estimates of the relative molar accumulation of the Mo-MuLV polypeptides seen in Fig. 2b, lanes A through D.

Effect of canavanine on the apparent mo-

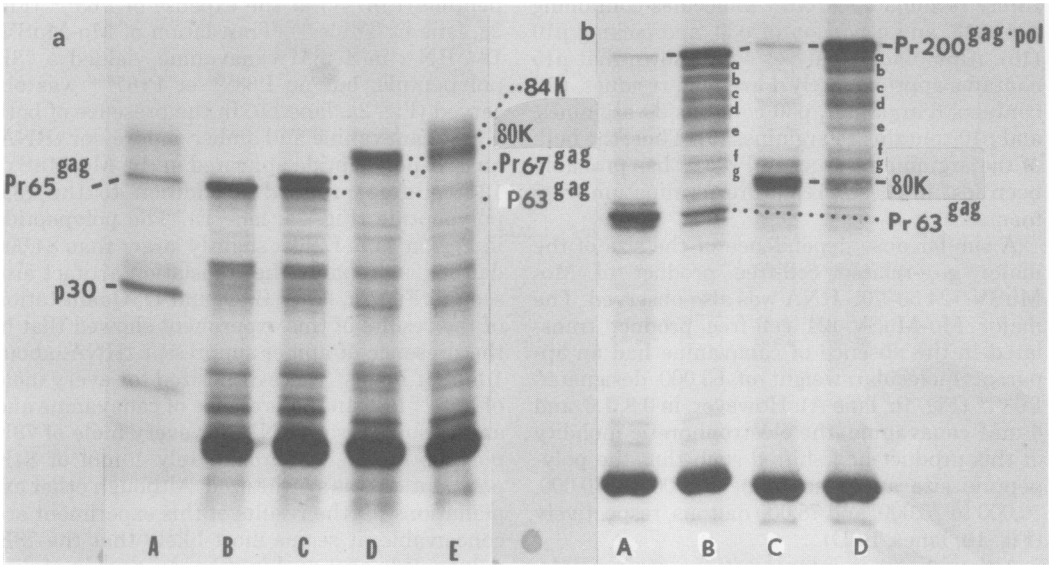

FIG. 2. *Combined effect of both canavanine and amber suppressor on the translation of Mo-MuLV and Mo-MuSV-124 RNA. (a) Effect of canavanine on the amber suppression of Mo-MuSV-124 polypeptide synthesis. The cell-free product of Mo-MuSV 124 30S RNA synthesized in the presence or absence of 4 mM canavanine and in the presence or absence of amber suppressor tRNA was analyzed by electrophoresis on 6 to 12% polyacrylamide gels. (Lane A) An R-MuLV polypeptide standard as described in Fig. 1; (lane B) Mo-MuSV-124 RNA, no canavanine, no suppressor; (lane C) Mo-MuSV-124 RNA, no canavanine, amber suppressor; (lane D) Mo-MuSV-124, 4 mM canavanine, no suppressor; (lane E) Mo-MuSV-124, 4 mM canavanine, amber suppressor. (b) Effect of canavanine on the amber suppression of Mo-MuLV polypeptide termination. The cell-free product of Mo-MuLV 35S RNA synthesized in the presence or absence of 4 mM canavanine and in the presence or absence of amber suppressor tRNA was analyzed by electrophoresis on 6 to 12% polyacrylamide gels. (Lane A) Mo-MuLV RNA, no canavanine, no suppressor; (lane B) Mo-MuLV RNA, no canavanine, amber suppressor; (lane C) Mo-MuLV RNA, 4 mM canavanine, no suppressor; (lane D) Mo-MuLV RNA, 4 mM canavanine, amber suppressor.*

TABLE 1. *Effect of canavanine on the amber suppression of Mo-MuSV-124 polypeptide biosynthesis*

Polypeptide	Polypeptide synthesized (mol)			
	Suppressor-minus tRNA	Amber suppressor tRNA	Canavanine	Canavanine plus amber tRNA
$P63^{gag}$	375[a]	178	None	None
$Pr67^{gag}$	31	231	None	None
78K	None	None	193	110
84K	None	None	None	66

[a] These data were obtained by dividing the radioactivity in a gel slice by the apparent molecular weight of the polypeptide. For the purposes of this analysis, an even distribution of methionine residues in the polypeptides was assumed.

TABLE 2. *Effect of canavanine on the amber suppression of Mo-MuLV polypeptide termination*

Polypeptide	Polypeptide synthesized (mol)			
	Suppressor-minus tRNA	Amber suppressor tRNA	Canavanine	Canavanine plus amber tRNA
$Pr200^{gag\text{-}pol}$	13.7[a]	237	20.6	372
$Pr80^{gag}$	None	None	898	393
$Pr70^{gag}$	228	261	None	None
$Pr63^{gag}$	914	464	203	168

[a] These data were obtained in the same way as described in Table 1.

lecular weights of ovalbumin and mengovirus-specific proteins. Since it appeared that canavanine incorporation artifactually increased the molecular weight of Mo-MuLV $Pr63^{gag}$, Mo-MuSV-124 $P63^{gag}$, and R-MuLV $Pr65^{gag}$, it seemed possible that this effect might be a general one. Thus, we examined the effect of canavanine on the cell-free product of ovalbumin and mengovirus 35S RNA. The results (Fig. 3 and 4) show that ovalbumin, which has a molecular weight of 43,000 when synthesized in the absence of canavanine (Fig. 3, lane B), shifted to an apparent molecular weight of 45,000 when synthesized in the presence of 4 mM canavanine (Fig. 3, lane C). The canavanine effect on mengoviral RNA translation was more complex. In

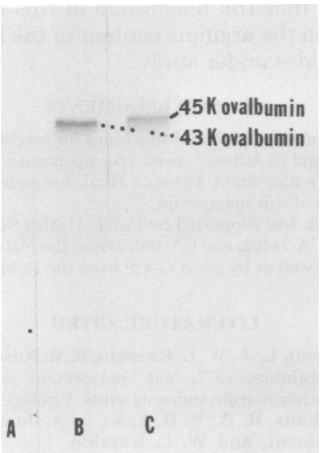

FIG. 3. *Effect of canavanine on cell-free ovalbumin synthesis. The cell-free product of polyadenylic acid-containing chick oviduct RNA synthesized in the presence or absence of 4 mM canavanine was immunoprecipitated with antiovalbumin, and the immunoprecipitates were analyzed on an 11.25% polyacrylamide gel. (Lane A) No RNA, no canavanine; (lane B) oviduct RNA, no canavanine; (lane C) oviduct RNA, 4 mM canavanine.*

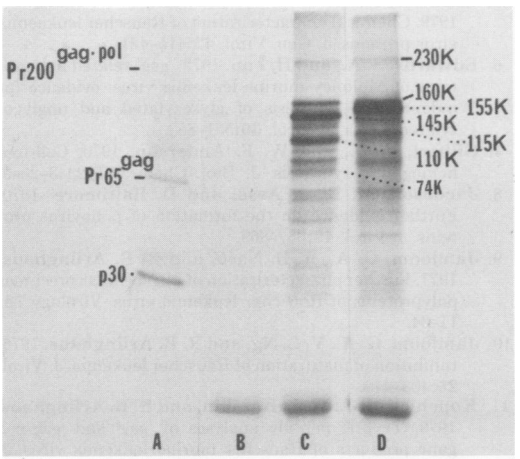

FIG. 4. *Effect of canavanine on the translation of mengoviral RNA. Mengovirus 35S RNA was translated in the presence or absence of 4 mM canavanine, and the cell-free product was analyzed on a 6 to 12% polyacrylamide gel slab. (Lane A) An R-MuLV protein standard; (lane B) no RNA, no canavanine; (lane C) mengoviral 35S RNA, no canavanine; (lane D) mengoviral 35S RNA, 4 mM canavanine.*

the presence of 4 mM canavanine, novel 155K and 115K polypeptides appeared in the mengoviral RNA cell-free product (Fig. 4, lane D), possibly at the expense of a 145K and a 110K polypeptide (Fig. 4, lane C). In addition, a 74K polypeptide disappeared (Fig. 4, lane C).

DISCUSSION

The arginine analog canavanine has been used in numerous studies dealing with murine retrovirus (2, 9, 10, 15, 22, 24, 26) and picornavirus (8) polypeptide formation. It has been generally accepted that canavanine exerts its effects by its incorporation into polypeptide chains (8) and subsequent interference with trypsin-like processing enzymes. In R-MuLV-infected cells, 3 to 4 mM canavanine causes the increased accumulation of an 80K polypeptide, initially designated $Pr80^{gag}$, at the apparent expense of $Pr65^{gag}$, the known precursor to the viral core proteins. Canavanine treatment, in addition, prevents the further cleavage of $Pr65^{gag}$ (2, 9, 15, 22, 24, 26) and efficiently blocks the release of newly formed virus (10). In R-MuLV-infected cells, a *gag*-related 80K polypeptide was observed in the absence of canavanine which had a half-life of about 15 min. Originally, the short-lived 80K polypeptide observed in cells not treated with canavanine and the 80K polypeptide which accumulated in the presence of canavanine were thought to be identical and an obligate precursor to $Pr65^{gag}$ (9). Recent findings, however, suggest that the 80K *gag*-related polypeptide which is seen in infected cells in the absence of canavanine is a glycosylated molecule (6, 23) and thus is probably not a precursor to $Pr65^{gag}$ and the core proteins. This polypeptide is now designated $gPr80^{gag}$. Recent experiments in our laboratory have confirmed that $gPr80^{gag}$ is glycosylated as measured by mannose incorporation and binding to lectin columns (Ng and Arlinghaus, unpublished data). In addition, it has been found that $gPr80^{gag}$ lacks a p30-characteristic tryptic peptide found in $Pr65^{gag}$ (11) and contains a mannose-labeled tryptic peptide not found in $Pr65^{gag}$ (Kopchick and Arlinghaus, manuscript in preparation).

The data presented in this report suggest that the $gPr80^{gag}$-like polypeptide which accumulates in cells and which is the major *gag*-related cell-free translation product of murine retrovirus RNA in the presence of canavanine is not $gPr80^{gag}$ but is in fact $Pr65^{gag}$ (or $Pr63^{gag}$) somehow altered by canavanine incorporation into the polypeptide chain such that it appears to have a molecular weight of 80,000. The dependence of the electrophoretic mobility of the *gag*-related cell-free product on the concentration of canavanine used strongly supports this view, as does the approximately 20,000-dalton upward shift of Mo-MuLV $Pr200^{gag\text{-}pol}$ and most of the polypeptides in the 80,000- to 200,000 molecular-weight range observed in the Mo-MuLV cell-free product synthesized in the presence of canavanine. This upward shift of $Pr200^{gag\text{-}pol}$ has also been seen in R-MuLV-infected cells treated

with canavanine (9, 10), but at the time this effect was attributed to an alteration in the processing of the $Pr200^{gag\text{-}pol}$ triplet (9).

Canavanine also appears to cause a small but quite clear shift in electrophoretic mobility of ovalbumin synthesized in its presence. This effect is not as dramatic as the shift of $Pr65^{gag}$ to 80,000 molecular weight, but may be explained by the fact that ovalbumin contains 3.1% arginine (14), as compared with 8.7% arginine for $Pr65^{gag}$ (5). Since ovalbumin is not known to be formed from a precursor nor does it appear to have an N-terminal leader sequence (18), the shift in electrophoretic mobility most probably does not reflect an actual change in the size of the polypeptide. Inclusion of canavanine in translations programed by mengovirus 35S RNA led to the accumulation of 155K and 115K polypeptides which were not seen in the absence of canavanine and did not appear to result from altered cleavage of mengoviral polypeptides (Murphy and Arlinghaus, unpublished data).

The results of cell-free translation of Mo-MuLV and Mo-MuSV-124 RNA in the presence of canavanine and amber suppressor tRNA provide a third line of evidence for the argument that canavanine incorporation causes a shift in polypeptide electrophoretic mobility. From a previous report (21), the results in this report, and another study (Murphy and Arlinghaus, submitted for publication), it seems clear that there is a suppressible UAG codon at the end of $P63^{gag}$, which in the Mo-MuSV-124 system allows the synthesis of $Pr67^{gag}$. In canavanine, a 78K polypeptide is made at the expense of $P63^{gag}$. Hence, it follows that $P63^{gag}$ and the 78K polypeptide most probably arise from the same mRNA and that synthesis of this 78K protein necessitates the read-through of the UAG codon at the end of $Pr63^{gag}$. Thus, if the 78K polypeptide synthesized in the presence of canavanine is really a precursor of $Pr63^{gag}$, amber suppressor should have no effect. However, if the 78K polypeptide is an altered $Pr63^{gag}$, amber suppressor tRNA should allow an additional 4,000 daltons to be added to the 78K polypeptide. Since an 84K polypeptide was observed to be synthesized in amounts proportional to the decrease in the 78K polypeptide when canavanine and amber suppressor tRNA were used in concert, we propose that the 78K polypeptide is in reality $Pr63^{gag}$ and that the 84K polypeptide is $Pr67^{gag}$.

Taken together, our results now lead us to the conclusion that although canavanine does prevent the cleavage of $Pr65^{gag}$ to the core proteins in infected cells (9, 10), it can also change the electrophoretic mobility of polypeptides. It is assumed that the magnitude of this effect will depend on the arginine content of the individual polypeptides under study.

ACKNOWLEDGMENTS

We are indebted to Shau-Ming Mong for excellent technical assistance and to James Syrewicz for maintenance of the cell cultures. We also thank Rebecca Bazer for assistance in the preparation of this manuscript.

This work was supported by Public Health Service grants CA-18248, CA-15495, and CA-16672 from the National Cancer Institute as well as by grant G-429 from the Robert A. Welch Foundation.

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