Detection of a Precursor-like Protein of Bovine Leukaemia Virus Structural Polypeptides in Purified Virions

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SUMMARY

Gel filtration chromatography of disrupted bovine leukaemia virus (BLV) resulted in the isolation of the 25,000 mol. wt. major internal protein (p25), two previously uncharacterized proteins of mol. wt. 65,000 (p65) and 12,000 (p12), and a mixture of p12 and a protein of mol. wt. 15,000 (p15). The p65 protein does not bind to concanavalin A and its antigenicity is ether resistant. Therefore, this polypeptide is different from the previously described glycoprotein associated with BLV. Radioimmunoprecipitation and competitive radioimmunoassays indicated that the p65 protein shares antigenic determinants with the p25, p15 and p12 proteins, respectively. Furthermore, tryptic peptide mapping demonstrated that p65 contains p25, p15, p12 and a BLV protein of mol. wt. 10,000 (p10). These results are consistent with the view that p65 is the precursor of gag gene-derived core proteins of BLV.

INTRODUCTION

Leukaemia (lymphosarcoma) in cattle is aetiologically associated with bovine leukaemia virus (BLV) (Ferrer et al. 1971, 1972; Stock & Ferrer, 1972; Ferrer et al. 1974) which is transmitted predominantly by contact (Piper et al. 1975; Ferrer et al. 1976b; Bech-Nielsen et al. 1978). BLV and its major internal antigen can be detected consistently in peripheral blood lymphocytes from infected cattle, but only after these cells have been cultured in vitro for several hours (Ferrer et al. 1972; Baliga & Ferrer, 1977).

BLV is morphologically similar to the C-type leukaemia viruses of other species (Ferrer et al. 1971; Stock & Ferrer, 1972) and like these viruses, BLV contains single-stranded 70S RNA (Callahan et al. 1976) and an RNA-dependent DNA polymerase or reverse transcriptase (Graves et al. 1977). On the other hand, BLV is immunologically different from all other known retroviruses. Evidence for a lack of antigenic relationship between the ether-resistant major internal antigen of BLV (p25) and the proteins of other mammalian leukaemia viruses was first reported in 1972 (Ferrer, 1972). Subsequently, the BLV p25 protein was purified to homogeneity and used to develop a sensitive and specific radioimmunoassay (RIA) (McDonald & Ferrer, 1976). Immunoprecipitation and competitive assays with radio-labelled BLV p25 confirmed that the BLV p25 protein does not possess any of the interspecies- or intraspecies-specific antigenic determinants demonstrated in other mammalian and avian retroviruses (Ferrer et al. 1976a; McDonald & Ferrer, 1976). It was also shown that the reverse transcriptase of BLV is antigenically unrelated to the analogous enzyme of other C-type viruses (Wuu et al. 1977). Furthermore, BLV, unlike any other known C-type
virus responsible for a natural occurring leukaemia, induces syncytia in cultures of non-transformed cells (Diglio & Ferrer, 1976; Ferrer & Diglio, 1976). These findings suggest that BLV is the prototype of a new family of leukaemia viruses and, therefore, an important model system for aetiological studies of leukaemia in other species.

An ether-sensitive glycoprotein of mol. wt. 51,000 to 69,000 (Devare & Stephenson, 1977; Frenzel et al. 1977) and a 15,000 mol. wt. polypeptide (p15) (Kaaden et al. 1977) have been purified from virus particles obtained from a BLV-infected foetal lamb kidney cell culture (FLK-BLV). Phillips et al. (1978) have also purified a 58,000 mol. wt. glycoprotein which is present in a soluble form in the supernatant fluid of cell line FLK-BLV. This culture is also infected with the bovine viral diarrhoea virus (BVDV) (communication from Dr M. J. Van der Maaten to all recipients of FLK-BLV culture, 11 July 1977), which is widespread in the cattle population and according to some investigators (Pritchett & Zee, 1975) has a buoyant density in sucrose similar to that of BLV. Thus, the identity of the virion polypeptides isolated from cell line FLK-BLV must be carefully evaluated.

In this paper we describe the isolation of several BLV polypeptides from purified BLV particles obtained from a culture which is free of BVDV and other adventitious agents. One of these proteins has a mol. wt. of 65,000 (p65) and has antigenic determinants in common with p25, p15 and the 12,000 mol. wt. protein (p12). Furthermore, the p65 protein was found to contain peptide sequences of these three virus proteins as well as of a BLV protein with a mol. wt. of 10,000 (p10). Thus, the p65 is analogous to the precursor protein found in other retrovirus systems.

**METHODS**

*Cells and virus.* The source of virus was a clone (clone 1) derived from cell line BLV-bat2 which was initiated by infecting a bat monolayer cell culture (TB_Lu; American Type Culture Collection, Rockville, Md., U.S.A.; CCL88) with BLV (Graves & Ferrer, 1976). As determined by electron microscopy, the RNA-dependent DNA polymerase assay (Graves et al. 1977) and the syncytia infectivity assay (Ferrer & Diglio, 1976), this cell line produced BLV continuously and abundantly. Repeated studies have shown that both cell line BLV-bat2 and clone 1 are free of adventitious agents, including BVDV and mycoplasma (Diglio & Ferrer, 1976; Ferrer & Diglio, 1976; Graves et al. 1977).

For the purification of BLV, clarified (4000 g, 15 min) supernatant fluids were collected from 4 to 7 day-old cultures of the BLV-bat2 clone 1 and centrifuged at 100,000 g for 2 h. The virus pellet was suspended in a small volume of TNE buffer (0.02 M-tris, 0.1 M- NaCl, 0.001 M- EDTA, pH 7.5) and centrifuged at 4°C for 2 h through a 20 to 60% discontinuous sucrose gradient in a SW27 rotor at 25,000 rev/min. Virus which banded at the 20 to 60% interface was further purified by two consecutive equilibrium sucrose density gradient centrifugations (McDonald & Ferrer, 1976; Graves et al. 1977).

*Sephadex chromatography.* Eight to ten mg of sucrose density gradient purified BLV was disrupted with 0.5% Triton X-100 and chromatographed on a 1 x 100 cm Sephadex G-150 column (Pharmacia Fine Chemicals, Piscataway, N.J., U.S.A.) equilibrated with buffer A (0.02 M-tris, 0.3 M- NaCl, 0.01 M- sodium azide, 0.01% Triton X-100, pH 7.8) at 4°C. The flow rate of the column was 8 to 10 ml/h. Fractions of 1 ml were collected and analysed by polyacrylamide gel electrophoresis (PAGE) and by immunodiffusion (ID) as described below. Fractions containing proteins with similar mol. wt. were combined, divided into aliquots and stored at −70°C. In most experiments the BLV proteins designated as p65, p25 and p12 were at least 90% pure after one single G-150 chromatography. In some cases, re-chromatography under the same conditions was necessary for final purification of these proteins. In the case of the p12 protein, re-chromatography over a Bio-Gel P30 column
Detection of a precursor-like protein in BLV

(1.5 × 35 cm, Bio-Rad Laboratories, Richmond, Calif., U.S.A.) under the same conditions was also found to be suitable for final purification. The control Rauscher murine leukaemia virus p30 protein was obtained from Dr J. T. August, Dept. of Molecular Biology, Albert Einstein College of Medicine, Bronx, N.Y., U.S.A.

Phosphocellulose chromatography. Phosphocellulose chromatography of labelled disrupted BLV or BLV p25 was performed as described by Devare et al. (1976). Approx. 0.5 × 10^6 to 2 × 10^6 ct/min radioactivity were applied to the column.

Concanavalin A chromatography. The concanavalin (Con-A) chromatography was performed on a 2 ml column containing Con-A bound to Sepharose 4B (Miles Laboratories, Elkhart, Ind., U.S.A.; Ponce de Leon et al. 1973). The column was washed with 0.01 M-phosphate buffer, pH 7.2 (PB) until no material with absorbance at 280 nm eluted from the column. Labelled protein was applied to the column and was washed exhaustively with PB until no radioactivity was detected in the eluate. The protein bound to the column was eluted with 0.1 M-α-methylmannoside in PB and radioactivity in the eluted fractions was measured in a gamma counter. The control bovine serum glycoprotein was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

Polyacrylamide gel electrophoresis. Each labelled BLV protein sample was mixed with standard unlabelled marker proteins. The mixtures were denatured at 100°C in buffer containing 0.0625 M-tris, 2% SDS, 5% mercaptoethanol, pH 6.8, for 2 min and analysed by 5 to 20% gradient SDS-PAGE (Mirault & Scherrer, 1971). The gels were then stained with Coomassie blue and destained by diffusion. The gel lanes marked by the stained protein bands were cut out and sliced into 2 mm strips and counted in a gamma counter.

Labelling of virus and virus proteins. Purified BLV disrupted with 1% NP-40, and BLV p25, p12 or the mixture of p12 and p15, were labelled with 125I by the chloramine T procedure (Hunter, 1967). The specific activities of the labelled p25 and p12 polypeptides were 2 × 10^4 to 4 × 10^4 and 1 × 10^4 to 2 × 10^4 ct/min/ng protein, respectively. The BLV p65 protein was radio-iodinated by an iodogen-catalysed reaction (Devare & Stephenson, 1977) to a specific activity of 2 × 10^4 ct/min/ng protein.

Immunological methods. Double antibody precipitation and competitive radioimmunoassays (RIA) were performed essentially as described by Strand & August (1973). Sera were titrated for binding activity with the labelled virus proteins as follows: each assay tube contained 60 μl RIA buffer (0.02 M-tris, 0.2 M-NaCl, 0.001 M-EDTA, 0.02% sodium azide, pH 7.5) with 2 mg bovine serum albumin (BSA) per ml, 10 μl of 4% NP-40 diluted in RIA buffer, 10 μl of 1:10 diluted normal serum from the same species as the test serum, 10 μl of labelled antigen (approx. 2 × 10^4 to 4 × 10^4 ct/min) and 10 μl of appropriately diluted test serum. The antigen and sera were diluted in RIA buffer containing 10 mg BSA per ml. The tubes were incubated for 3 h at 37°C and then overnight at 4°C; thereafter the antigen–antibody complexes were precipitated with an excess of antisera to the γ-globulin of the test serum. After 1 h incubation at 37°C followed by 3 h at 4°C, 0.5 ml of RIA buffer was added to each tube and the samples were centrifuged at 2000 g for 25 min. Supernatants were removed and the pellets were washed twice with 0.5 ml buffer. The radioactivity in the pellet was determined in a gamma counter.

The competitive RIA was initiated with a dilution of the test serum capable of binding 50% of the labelled antigen. Competing antigens were diluted in RIA buffer containing 10 mg BSA per ml and were added (10 μl) immediately before the antiserum. All other steps of the RIA were performed as described above.

Immunodiffusion (ID) tests were carried out in 0.7% Noble special agar (Miller & Van der Maaten, 1976). Control BLV p25 antigen was partially purified by DEAE-cellulose chromatography as follows: 3 to 5 mg of purified Triton X-100 disrupted BLV was applied
to a DEAE-cellulose (Whatman, H., Reeve Angel and Co., Clifton, N.J., U.S.A.) column (1.5 × 6 cm) equilibrated with 0.02 M-tris, pH 8.3. The column was washed with the same buffer and p25 was eluted at a salt concentration of 0.05 M-NaCl. The protein was dialysed and then concentrated by lyophilization. When tested by ID with the reference BLV sera, the control antigen formed a single precipitation line which gave a reaction of complete identity with the precipitin line formed by the BLV p25 antigen isolated in previous studies (McDonald & Ferrer, 1976).

Reference BLV sera Se-276 and 27-125 were obtained from a leukaemic cow and a regression case of bovine leukaemia, respectively. These sera have high titres of antibody against BLV p25 and other BLV antigens (Diglio & Ferrer, 1976; Ferrer et al. 1976a; McDonald & Ferrer, 1976; Wuu et al. 1977). Control serum Se-382 was obtained from a cow free of BLV infection. This serum was negative for antibodies to BLV as determined by immunofluorescence (Ferrer et al. 1972), ID (Ferrer et al. 1972; Miller & Van der Maaten, 1976), radioimmunoprecipitation (McDonald & Ferrer, 1976) and virus neutralization (Ferrer & Diglio, 1976; Ferrer et al. 1977) tests.

Monospecific anti-p25 serum was prepared in a rabbit by immunization with BLV p25 obtained by G-150 chromatography following the procedure described above. As determined by SDS-PAGE, the immunizing antigen was at least 90% pure. The rabbit anti-p25 serum reacted strongly in ID with purified p25, but not with the BLV-associated glycoprotein described by Miller & Van der Maaten (1976) nor with BLV p12 or the mixture of BLV p12 and p15 obtained in the present study. On the other hand, all of these BLV antigen preparations reacted with the broadly reactive reference BLV bovine serum 27-125. Rabbit anti-BSA serum was purchased from Miles Laboratories.

**Tryptic peptide mapping.** Two hundred µg of unlabelled purified BLV was solubilized by 2% SDS and 5% mercaptoethanol and then electrophoresed on a 8.5 to 20% gradient polyacrylamide gel in the presence of SDS as described above. Purified p65 was electrophoresed under the same conditions. After staining and destaining, stained protein bands corresponding to mol. wt. of 65,000, 25,000, 15,000, 12,000 and 10,000 were cut out, washed and radio-iodinated with 125I as described by Elder et al. (1977).

The labelled protein bands were dried by lyophilization, re-hydrated with 0.5 ml of 0.05 M-ammonium carbonate buffer, pH 8.0, containing 50 µg/ml of TPCK trypsin (Worthington Biochemicals Corp., Freehold, N.J., U.S.A.) and then incubated at 37°C overnight. The supernatant fluids containing the soluble tryptic peptides were removed and lyophilized. Radioactivity (2 × 10⁶ to 4 × 10⁶ ct/min) recovery from the gel varied from 60 to 80%. The lyophilized peptides were dissolved in 20 µl of buffer I (acetic acid:formic acid:H₂O, 15:5:80) and 1 to 5 µl (1 × 10⁶ to 2 × 10⁶ ct/min) of this mixture were resolved by two-dimensional fingerprinting on cellulose-coated thin-layer plates (10 by 10 cm) as described by Elder et al. (1977). The plates were then dried and exposed to Kodak X-Omat X-ray film with screen at −70°C.

**RESULTS**

Phosphocellulose chromatography of 125I-labelled disrupted BLV was initially applied to determine the optimum conditions for the purification of the BLV proteins. However, in repeated attempts it was found that, under the conditions used, the BLV proteins, unlike the proteins of other retroviruses (Strand & August, 1973; Barbacid et al. 1976a), did not bind significantly to phosphocellulose. Gel filtration chromatography was then employed to isolate the BLV proteins.

Disrupted BLV was chromatographed on a G-150 column under non-denaturing conditions and the eluted fractions were analysed by SDS-PAGE and by ID with bovine
Detection of a precursor-like protein in BLV

Fig. 1. SDS-PAGE of $^{125}$I-labelled BLV structural proteins isolated by G-150 chromatography. Approx. $1 \times 10^6$ ct/min of each polypeptide was applied on the gel: (a) p65, (b) p25, (c) p12, and (d) a mixture of p12 and p15. An autoradiogram of $^{125}$I-labelled disrupted BLV electrophoresed under identical conditions is shown on the top of the figure. The positions of marker proteins for the calibration of mol. wt. are indicated by arrows.

reference sera Se-276 (or 27-125) and control p25 antigen. P25 antigenic activity was detected in a wide range of eluted fractions ($Ve/Vo = 1.6$ to 2.3). In some of these fractions ($Ve/Vo = 1.6$ to 1.8) the SDS-PAGE analysis showed a single polypeptide of mol. wt. 25000. However, in few other fractions which also had p25 antigenic activity, the SDS-PAGE analysis failed to demonstrate a 25000 mol. wt. polypeptide. Instead, these fractions ($Ve/Vo = 2.15$) contained a protein with mol. wt. 65000 (p65). These p65 containing fractions were used for subsequent studies on the p65 polypeptide. In the fractions which did not
Table 1. Radioimmunoprecipitation of \(^{125I}\)-BLV p65 protein

<table>
<thead>
<tr>
<th>Serum</th>
<th>(^{125I})-p65 immunoprecipitated*</th>
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<tr>
<td>Reference BLV bovine serum Se-276</td>
<td>30 250 100</td>
</tr>
<tr>
<td>Reference BLV bovine serum 27-125</td>
<td>25 584 85</td>
</tr>
<tr>
<td>Negative bovine serum Se-382</td>
<td>3589 12</td>
</tr>
<tr>
<td>Rabbit antiserum to BLV p25</td>
<td>25 624 85</td>
</tr>
<tr>
<td>Normal rabbit serum</td>
<td>2134 7</td>
</tr>
<tr>
<td>Rabbit antiserum to BSA</td>
<td>3132 11</td>
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</tbody>
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* Input radioactivity was \(3.7 \times 10^4\) TCA-precipitable ct/min.

have p25 antigenic activity, we found a single polypeptide of mol. wt. 12,000 (p12). A mixture of p12 and a protein of mol. wt. 15,000 (p15) were also detected in fractions which were free of p25 antigenic activity. Both p12 and the mixture of p12 and p15 reacted in ID with the broadly reactive bovine reference BLV serum 27-125, but not with the anti-p25 serum. Based on the relative yield of the isolated proteins we estimate that p65 content in the purified virion is approx. one-third that of p25.

For further evaluation of purity, the isolated BLV proteins were labelled with \(^{125I}\) and analysed by SDS-PAGE. The results are presented in Fig. 1. The upper part of the figure shows an SDS-polyacrylamide gel autoradiogram of \(^{125I}\)-labelled disrupted BLV. The electropherograms in the lower part of Fig. 1 show that p65, p25 and p12 migrated as single homogeneous peaks in their corresponding mol. wt. regions; thus, these polypeptides were considered to be more than 90% pure. Fig. 1 shows that the mixture of p12 and p15 did not contain other detectable proteins. Purified radio-labelled p65 (\(^{125I}\)-p65) and p25 (\(^{125I}\)-p25) also migrated as single homogeneous components when analysed by PAGE in the presence of 8 M-urea at pH 3.8 (Duesberg et al. 1968; our results not shown). This observation argues strongly against the possibility that p65 and p25 are aggregates of smaller polypeptides. In two separate experiments it was found that less than 5% of \(^{125I}\)-p25 bound to phosphocellulose columns. This was in agreement with the results of our earlier unsuccessful attempts to separate the virion proteins by phosphocellulose chromatography of radio-labelled disrupted BLV.

The results summarized in Table 1 show that 80% or more of the \(^{125I}\)-p65 protein was immunoprecipitated by the reference BLV bovine serum Se-276. This protein was also effectively precipitated by bovine serum 27-125 or by the rabbit anti-p25 serum. In contrast, no significant precipitation was observed with the control negative sera or the rabbit antiserum against BSA. The titration patterns of the positive sera were linear in the range of 30 to 80% precipitation. Thus, the results of the radioimmunoprecipitation indicate that the BLV p65 protein has the antigenic determinant(s) normally found in the p25 molecule.

Competitive RIAs were carried out to confirm this finding and to determine whether p65 also has determinants in common with p12 and p15. The assays were conducted with purified \(^{125I}\)-p65 antigen and a dilution of bovine serum Se-276 that bound 50% of this antigen. Unlabelled competing antigens were p65, p25, p12 and the p12-p15 mixture, all of which were tested in amounts ranging from 1 to 80 ng. As shown in Fig. 2, 80 ng of p65 displaced more than 80% of the iodinated antigen and the slope of the curve was linear in the range of 10 to 80% displacement. The other BLV proteins also significantly competed with \(^{125I}\)-p65 antigen, but to a lesser degree. Maximum competition was 40% with p12 and 60% with either p25 or the p12-p15 mixture. No competition was observed with control p30 from the Rauscher murine leukaemia virus (R-MuLV). The slopes of the curves indicate that, at lower concentrations, p25 and the p12-p15 mixture competed more efficiently than p65.
Detection of a precursor-like protein in BLV

Fig. 2. Competitive RIA for the BLV p65 protein with reference BLV bovine serum Se-276. The assays were performed with 2 ng of $^{125}$I-p65 (approx. $4 \times 10^4$ ct/min) and a 1:2000 dilution of Se-276 serum. □—□, R-MuLV p30; ●—●, p12; ■—■, p12 + p15; ▲—▲, p25; △—△, p65.

Fig. 3. Competitive RIA for the BLV p25 proteins with the rabbit anti-p25 serum. The assay was performed with 1 ng of $^{125}$I-p25 (approx. $2.5 \times 10^4$ ct/min) and a 1:4000 dilution of rabbit anti-p25 serum. ●—●, p12; ■—■, p12 + p15; ▲—▲, p25.

This suggests that the antigenic reactivity of the p25 and p15 determinants towards their respective antibodies are greater in the mature proteins than in the p65 polypeptides.

The possibility that the competing activity of p12 and p15 with $^{125}$I-p65 was due to contaminating p25 can be ruled out. Indeed, p12 and the p12–p15 mixture failed to compete with the $^{125}$I-p25 antigen in assays conducted with rabbit anti-p25 serum (Fig. 3). As shown in Fig. 4, p65 competed efficiently in the RIA for $^{125}$I-p25 (Fig. 4a) and in the RIA for $^{125}$I-
Fig. 5. Two-dimensional tryptic peptide maps of $^{131}$I-labelled p65 and virus core proteins. The labelled proteins were digested with TPCK-trypsin and separated on cellulose thin-layer plates in the first dimension by electrophoresis (left to right), followed by ascending chromatography. Approx. $1 \times 10^5$ to $4 \times 10^6$ cpm/min of each tryptic digest was applied to the thin-layer plate. The plates were exposed to X-ray film for 3 to 18 h. In reconstitution experiments equal volumes of labelled peptides from BLV p25, p15, p12 and p10 were mixed before electrophoresis and separated on a thin-layer plate as described above. (a) p65, (b) p25, (c) p15, (d) p12, (e) p10 and (f) a mixture of p25, p15, p12 and p10.
Detection of a precursor-like protein in BLV

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p12 (Fig. 4b). These results further demonstrate the presence of antigenic determinants of p25 and p12 in the p65 protein.

To establish further the relationship of p65 to other BLV structural proteins, two-dimensional tryptic peptide maps of radio-iodinated p65, p25, p12, p15, as well as p10, were performed. Analysis of the results (Fig. 5a to e) indicate that p65 contained most of the tyrosine-containing tryptic peptides of p25 (spots 1 to 6), p15 (spots 7 and 8), p12 (spots 9 to 16) and p10 (spots 17 to 34). The pairs of peptides 6 and 22, 11 and 21, as well as 12 and 29, are not resolved in the peptide map of p65 (Fig. 5a). We noticed, however, that the p65 protein contained tryptic peptides (spots 35 to 39) that are not present in other BLV structural proteins. One may speculate that these extra peptides could be generated from the segment of the p65 molecule that is not shared by the p25, p15, p12 or p10 proteins. The presence of large numbers of labelled peptides in the p10 protein is rather unusual for a polypeptide of relatively low mol. wt. This suggests that p10 may be very rich in tyrosine and lysine or arginine residues. Reconstitution experiments in which p25, p15, p12 and p10 were mixed and analysed by two-dimensional fingerprinting confirmed the relative positions of the peptides of BLV structural protein in the p65 polypeptide (Fig. 5f). The BLV p65 protein did not bind to Con-A under conditions in which a control bovine glycoprotein fraction bound efficiently (results not shown).

In another experiment (not shown) an aliquot of labelled BLV p65 protein (25 x 10^4 ct/min) was treated with 2 vol. of ether for 2 h. After the removal of ether, the treated protein, along with the untreated control p65, were tested by radioimmunoprecipitation with reference serum Se-276. The results of these experiments showed that the antigenicity of BLV p65, unlike that of the BLV-associated glycoprotein (Ferrer et al. 1976a; Onuma et al. 1976), was not affected by the ether treatment.
DISCUSSION

Using gel filtration in Sephadex G-150, p25 and two previously uncharacterized BLV proteins, p65 and p12, have been isolated from purified virions. The p65 protein does not bind to Con-A and its antigenicity is ether resistant. Thus, this polypeptide is clearly different from the glycoprotein of a similar mol. wt. detected in virus particles and supernatant fluids obtained from BLV-producing cell cultures (Ferrer et al. 1976a; Miller & Van der Maaten, 1976; Onuma et al. 1976; Devare & Stephenson, 1977; Frenzel et al. 1977; Phillips et al. 1978).

It has been shown that the structural proteins of several retroviruses are processed in vivo from large precursor polypeptides by post-translational cleavage (Vogt & Eisenman, 1973; Vogt et al. 1975; Barbacid et al. 1976b; Arcement et al. 1977; Okasinski & Velicer, 1977). The precursors of the internal (gag) proteins of these retroviruses have mol. wt. ranging between 60,000 and 80,000 and share antigenic determinants and tryptic peptide sequences with the mature proteins. The results of the radioimmunoprecipitation tests and competitive RIA presented in this paper clearly demonstrate that BLV p65 has antigenic determinants of p25, p15 and p12. Furthermore, two dimensional tryptic peptide mapping demonstrated that the BLV proteins p25, p15, p12, as well as p10, are contained in the p65 polypeptide. Thus, it seems clear that the p65 is an uncleaved precursor of the BLV core proteins. Recently, it has been shown that a polypeptide of mol. wt. 70,000 is synthesized and correctly cleaved into virion proteins p25, p15 and p10 in Xenopus laevis oocytes microinjected with BLV 38S RNA (Ghysdael et al. 1979). It seems likely that this 70,000 mol. wt. protein corresponds to BLV p65.

The precursor proteins of other retroviruses have been demonstrated in infected cells and only occasionally in virus particles (Jamjoom et al. 1975; Oskarsson et al. 1975; Okasinski & Velicer, 1977). Usually the amount of precursor proteins detected in virions is relatively small. In contrast, our results show that BLV particles contain relatively large quantities of p65. Indeed, if we assume that p25 comprises 30% of the proteins of BLV, then p65 content could be as much as 10% of the total virion proteins. This suggests a deficient cleavage of the precursor polypeptide in the BLV-infected bat cells due perhaps to low levels of specific proteases or to a defective cleavage site.

It has been reported that avian and murine retroviruses contain a proteolytic factor which specifically cleaves the gag precursor polyprotein (Von der Helm, 1977; Yoshinaka & Luftig, 1977a), and that the maturation of virus cores is accomplished by this process (Yoshinaka & Luftig, 1977b). Therefore, the morphological features that distinguish BLV from the typical C-type viruses (Ferrer et al. 1972; Stock & Ferrer, 1972; Calafat et al. 1974) could conceivably be attributed to the impaired cleavage of p65. Indeed, it has been suggested that the structural abnormalities observed in the feline leukaemia virus pseudotype of the mouse sarcoma virus may be explained by the presence in the mature virions of a large polypeptide (Oskarsson et al. 1975).

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Detection of a precursor-like protein in BLV

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