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EXPRESSION OF BOVINE LEUKAEMIA VIRUS ANTIGENS FUSED TO MS2 POLYMERASE IN *E. COLI*

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Summary. - The main core protein and segments of envelope proteins of bovine leukaemia virus fused to MS2 polymerase were expressed in *E. coli*. The synthesis rate varied between 3 and 25 % of the total cellular proteins. BLV-MS2 polymerase fusion proteins were detected immunologically using rabbit anti-BLV sera, monoclonal antibodies and a serum of a BLV-infected cow.

Key words: bovine leukaemia virus; microbially synthesized antigens; fusion proteins; diagnosis of enzootic bovine leukosis

Introduction

The diagnosis of BLV infection is based on the serological detection of antibodies directed against structural proteins. For preparation of BLV antigens, cultures of BLV-infected mamalian cells are used. An alternative way is the expression of these proteins in *E. coli*.

The expression of BLV-β-galactosidase fusion proteins containing different segments of *gag* or *env* gene has been reported previously (Zajac and Slávikovà, 1989; Zajac *et al.*, 1989; Ulrich *et al.*, 1990*a*). The fusion proteins carrying p24, gp30 or the short gp51 segment reacted strongly with sera of BLV-infected cattle demonstrating their principal suitability for diagnosis of BLV infection (Siakkou *et al.*, 1990).

To test the possibility of increasing the expresion level of the BLV segments and to reduce the cross reactivity of bovine sera with the prokaryotic part of fusion protein we attempted to employ a plasmid derivatives of pPLc 24 (Remaut *et al.*, 1981, 1983*a, b,*) modified by Seedorf *et al.* (1987).

Materials and Methods

Plasmids and bacteria. E. coli K12 host strains NF1 (Δ H1 Δ trp lacZ$^-$am carrying a defective λ prophage N am7 N am53 cI857 Δ H1; Bernard *et al.*, 1979) and pop 2136 (Haymerle *et al.*, 1986)

were kind gifts of Dr. K. K. Stanley (EMBO Lab. Heidelberg) and of Dr. Raibaud (Pasteur Institute, Paris).

The pPLc24-derived vectors pEx 8-mer, pEx 10-mer, and pEx 12-mer encoding 100 amino acids of MS2 polymerase (Seedorf *et al.*, 1987) were kindly provided by Dr. K. Seedorf. In this work they are designated pMS2 vectors.

The pUC subclones of the BLV genome were described earlier (Ulrich *et al.*, 1990*a*).

Sera. Monoclonal antibodies directed against gp51 and p24 epitopes were prepared by C. Platzer (Platzer *et al.*, 1990). Anti-BLV serum was obtained from rabbits inoculated with purified BLV lysate. The serum of a BLV-infected cow was serologically characterized by the occurrence of antibodies directed against gp51, gp30, and p24.

Cloning and expression. Cloning procedures were as described by Maniatis *et al.* (1982). The cloning strategy of BLV DNA fragments isolated from proviral genome HU1 (Nyakatura *et al.*, 1985) into pMS2 vectors was similar to that used for insertion into pEX vectors (Ulrich *et al.*, 1990*a*). The recombinants were marked by „/pMS2" (or „/pEX"). SDS polyacrylamide gel electrophoresis and Western blotting were performed according to Laemmli (1970) or Towbin *et al.* (1979), respectively.

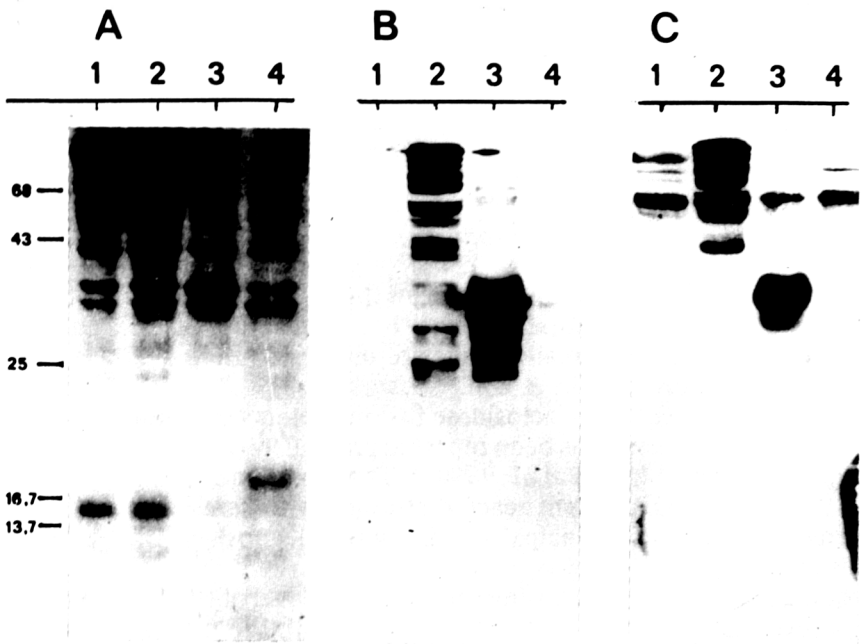

Fig. 1

Detection of the expression of p24 fusion proteins using 10 % Coomassie blue-stained polyacrylamide gel (A), Western blotting using mouse monoclonal antibody (B) or serum of a BLV-infected cow (C)

Lysates of approximately 10^7 cells expressing β-galactosidase (pEX: lane 1), p24-β-galactosidase fusion protein (pGAG1/pEX: lane 2), p24-MS2 polymerase fusion protein (pGAG1/pMS2: lane 3) and MS2 polymerase segment (pMS2: lane 4) were applied to the gels.
Positions of molecular size standards are noted on the left side (in kD).

Results

A DNA fragment encoding 72 % of the main core protein p24 (amino acids 46–199) was cloned using an *Eco*RI-*Hind*III fragment from pGAG1/pEX (Ulrich *et al.*, 1990*a*) generating pGAG1/pMS2. Plasmid pGAG1/pMS2 expressed a fusion protein (approximately 37 kD) which reacted whit rabbit anti-BLV serum, a monoclonal antibody directed against p24 and a serum of a BLV-infected cow (Fig. 1). The expression rate was estimated to 20–25 % of the total cellular proteins, the p24 part corresponding to 12–14 % of the total cellular proteins.

The expression of a *Bcl*I- DNA fragment encoding amino acids 135–268 of mature gp51 and 1–45 of gp30 and the reactivity of the expressed MS2 polymerase fusion protein has already been reported (pENV4/pMS2; Ulrich *et al.*, 1990*a*). In lysates of cells transformed by pENV4/pMS2 two different-sized fusion proteins (30 kD, 32 kD) were detected by all antisera used. In Coomassie blue-stained gels only the smaller one was observed (5–10 % of the total cellular proteins).

The *env* DNA fragments encoding amino acids 56–103 of mature gp51 and amino acids 45–195 of gp30 were inserted into pMS2 vectors (pENV1/pMS2, pENV5/pMS2) using the respecive *Bgl*II- *Bam*HI and *Bcl*I-*Bgl*II restriction

Table 1. Expression rates of BLV proteins fused to MS2 polymerase in comparison to those fused to β-galactosidase*

Recombinant plasmid	Coded sequence (aa of mature protein)	Expression rates / % of total cellular protein[a]	
		fusion protein	BLV derivative
pGAG1/pMS2	p24 (46-199)	20–25	12–14
pGAG1/pEX	p24 (46-199)	20–25	2.5–3
pENV1/pMS2	gp51 (56-103)	< 1[b]	< 0.5
pENV1/pEX	gp51 (56-103)	20–25	0.8–1
pENV4/pMS2	gp51 (135-268) gp30 (1-45)	5–10	3–6
PENV4/pEX	gp51 (135-268) gp30 (1-45)	5	0.6
PENV5/pMS2	gp30 (45-195)	3–5	2–3
PENV5/pEX	gp30 (45-195)	8	1

[a] calculated from Coomassie blue-stained gels by scanning
[b] only detected in Western blot using monoclonal antibodies
* Ulrich *et al.*, 1990*a*

fragments. The fusion protein encoded by pENV1/pMS2 was expressed at very low level. It was detected only in Western blots using a monoclonal antibody (not shown). The gp30-encoding clone pENV5/pMS2 synthesized fusion proteins with molecular weights of 29 kD and 27 kD. The reactivity of the larger protein corresponding to 3–5 % of the total cellular proteins, with the rabbit and cattle serum was more pronounced (not shown).

Discussion

A comparison of the expression rates of BLV proteins fused to β-galactosidase (Ulrich *et al.*, 1990*a*) or 100 amino acids of MS2 polymerase (this work) revealed only slight differences between those encoded by pGAG1, pENV4, and pENV5 (3–25 % of the total cellular proteins, Table 1). In both systems p24 fusions were found in the largest amounts. Because of the smaller procaryotic part in MS2 polymerase fusion proteins, the amount of BLV proteins was 2–5 fold increased as compared to the pEX expression system. The expression of the short gp51 segment (amino acids 56–103) was drastically decreased when fused to MS2 polymerase with respect to the β-galactosidase fusion. Very low expression level was also observed in N-terminal fusion with coat protein of phage fr (Kozlovskaya *et al.*, 1986). By shortening the BLV fragment the synthesis was increased (Ulrich *et al.*, 1991). On the other hand, the C-terminal fusion of the BLV segment to hepatitis core antigen leads to a high-level expresion (Borisova *et al.*, 1989; Ulrich *et al.*, 1990*b*). The reasons for these particularities have not been studied in detail but differences in protein stability are expected to play a role.

Cattle sera have been found to contain high titres of antibodies directed against bacterial β-galactosidase (Siakkou *et al.*, 1990). We have demonstrated the absence of antibodies reacting with MS2 polymerase 100 amino acid segment in a cow serum. This represents an improvement of the conditions for a diagnostic use of the fusion proteins.

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