

Purification of Murine and Feline Type-C Virus Envelope Polypeptides as Micellar Protein Complexes

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Mammalian Type-C Viruses, Envelope Polypeptides, Purification, Micellar Complexes, Peptide Maps

A technique originally described for the isolation of Friend leukaemia virus envelope polypeptides [1] yields equivalent structures from Moloney leukaemia, AKR and BALB/c xenotropic virus as well as feline leukaemia virus. The envelope polypeptides are obtained as micellar protein complexes, named rosettes. Rosettes of the five mammalian type-C viruses examined are indistinguishable by electron microscopy. Separation of these aggregates in polyacrylamide gel electrophoresis under nonreducing conditions reveals a glycoprotein of about 85000 d as their major component. Tryptic peptide analyses identify the viral origin of these polypeptides and emphasize strain specific differences in their primary structure.

Env-gene products of type-C retroviruses perform such general functions as adsorption to and penetration of the host cell membrane. These viral polypeptides express distinct classes of antigenic determinants [2]. To these the natural host may respond immunologically [3] leading to virus neutralisation, elimination of virus producing cells and perhaps the regression of virus-induced tumor [4, 5]. Moreover evidence has accumulated that env-gene products influence, if not determine, important biological peculiarities of type-C viruses. These comprise supposed physiological functions of endogenous proviral sequences [6, 7] as well as oncogenicity which some viruses seem to have acquired by recombination involving their env-genes [8, 9].

For a more detailed understanding of these biological phenomena a deeper insight into the biochemical structure of the viral envelope is needed. Such investigations usually require large amounts of homogeneous material. Thus we have elaborated a relatively simple and efficient procedure for the isolation of envelope polypeptides from Friend leu-

kaemia virus (FLV) [10, 1]. Here we demonstrate that this technique yields comparable preparations from other type-C viruses as well.

The knob-like envelope projections of mammalian type-C retroviruses [12] are probably composed of 4–6 [13] glycoprotein molecules of about 85 kd (gp 85) [14]. In general gp 85 consists of two polypeptide chains gp 71 and p 15E. The hydrophilic gp 71 is anchored to the membrane polypeptide p 15E partly by disulfide bridges, the rest is noncovalently associated in the same fashion. The noncovalently linked fraction of gp 85 can be transferred almost quantitatively into the covalently linked form of gp 85 by treatment with sulfhydryl activating reagents such as 2,2'-dithiobis(m-nitropyridine) or N-ethylmaleimide [15]. When the viral membrane is solubilised with Triton X-100 the amphiphilic gp 85 forms micellar protein complexes, named rosettes [10]. Since rosettes are soluble in aqueous solution they can be separated from the rest of the virus by velocity sedimentation. Rosettes are multivalent and thus are particularly suited for receptor studies [16] and vaccination against virus-induced leukaemia [17]. If desired these 32S complexes can be separated into their constituents gp 71 and p 15E by molecular sieve chromatography [1].

As demonstrated in Fig. 1 this protocol yields results comparable to those obtained with FLV for Moloney leukaemia virus (MoLV), exogenous AKR (AKR), xenotropic BALB/c (B-MuX) and feline leukaemia virus (FeLV). Analyses of these complexes in polyacrylamide gel electrophoresis (PAGE) under nonreducing conditions reveal a predominant polypeptide component migrating to the position of about 85 kd (Fig. 1a). The electron microscopic examination of preparations from the five viral isolates shows structures very similar to FLV rosettes shown in Fig. 1c. They are round to ovoid particles (diameter 16 ± 3 nm) with a fuzzy surface. Viral knobs are smaller, more homogeneous in size (diameter 10 ± 1 nm) and occasionally subunits appear to be visible (Fig. 1b).

Evidence for the viral origin of the 85 kd component contained in rosettes was obtained by tryptic peptide analyses (Fig. 2). Rosettes were separated on analytical PAGE under reducing and nonreducing conditions. The 85 kd band disappeared after reduction. A new band is seen in the 70 kd region and the 15 kd polypeptide is more prominent (not shown). Tryptic peptide maps of the 85 kd and the 70 kd polypeptide

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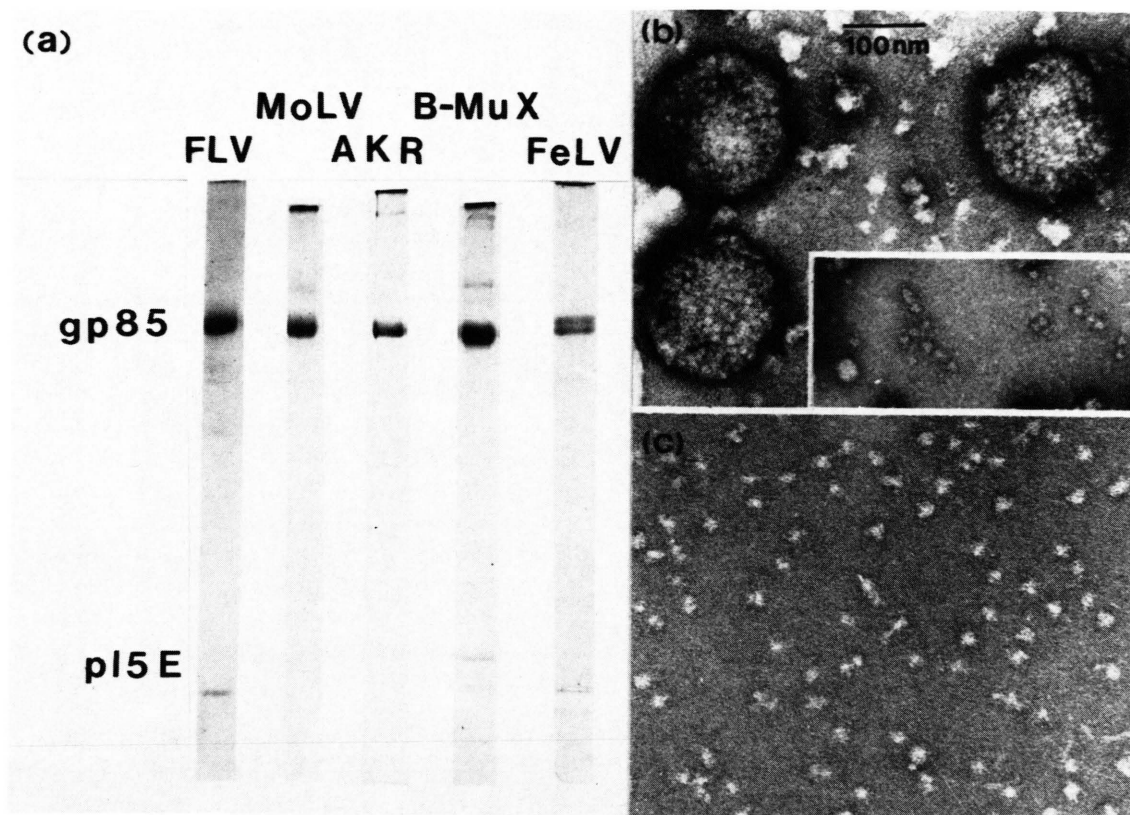


Fig. 1. PAGE analysis and electron microscopy of rosettes from various mammalian type-C viruses. MoLV was harvested from Sac(+) cells [11]. B-MuX virus producing SIRC cells were obtained from Dr. Moroni, Friedrich-Miescher-Institute, Basel. The origin of AKR and FLV-producing cells have previously been described [14]. FeLV was propagated in FL-74, a cell line kindly provided by G. Theilen, Davis, California. Rosettes were prepared according to our published procedure [1] and analysed in PAGE (9–16% acrylamide gradient) under nonreducing conditions. Proteins were stained with Coomassie blue (a). The electron micrographs show uranyl acetate stained FLV (b), detached viral knobs (insert, b) and FLV rosettes (c).

of the same virus are very similar. However, with the exception of MoLV maps the 85 kd components reveal minor additional spots not seen on maps of the 70 kd polypeptide. As we have shown earlier these additional spots are specific for p15E [1]. The maps of Fig. 2 display the characteristic patterns published by Elder *et al.* [18, 8] for individual murine type-C virus glycoproteins. Thus the main protein component of rosettes is identified as viral gp85. Contaminating high molecular weight polypeptides in some preparations of rosettes, are probably of cellular origin. Their pattern of tryptic peptides are completely different from gp85 and p30 virion polypeptides.

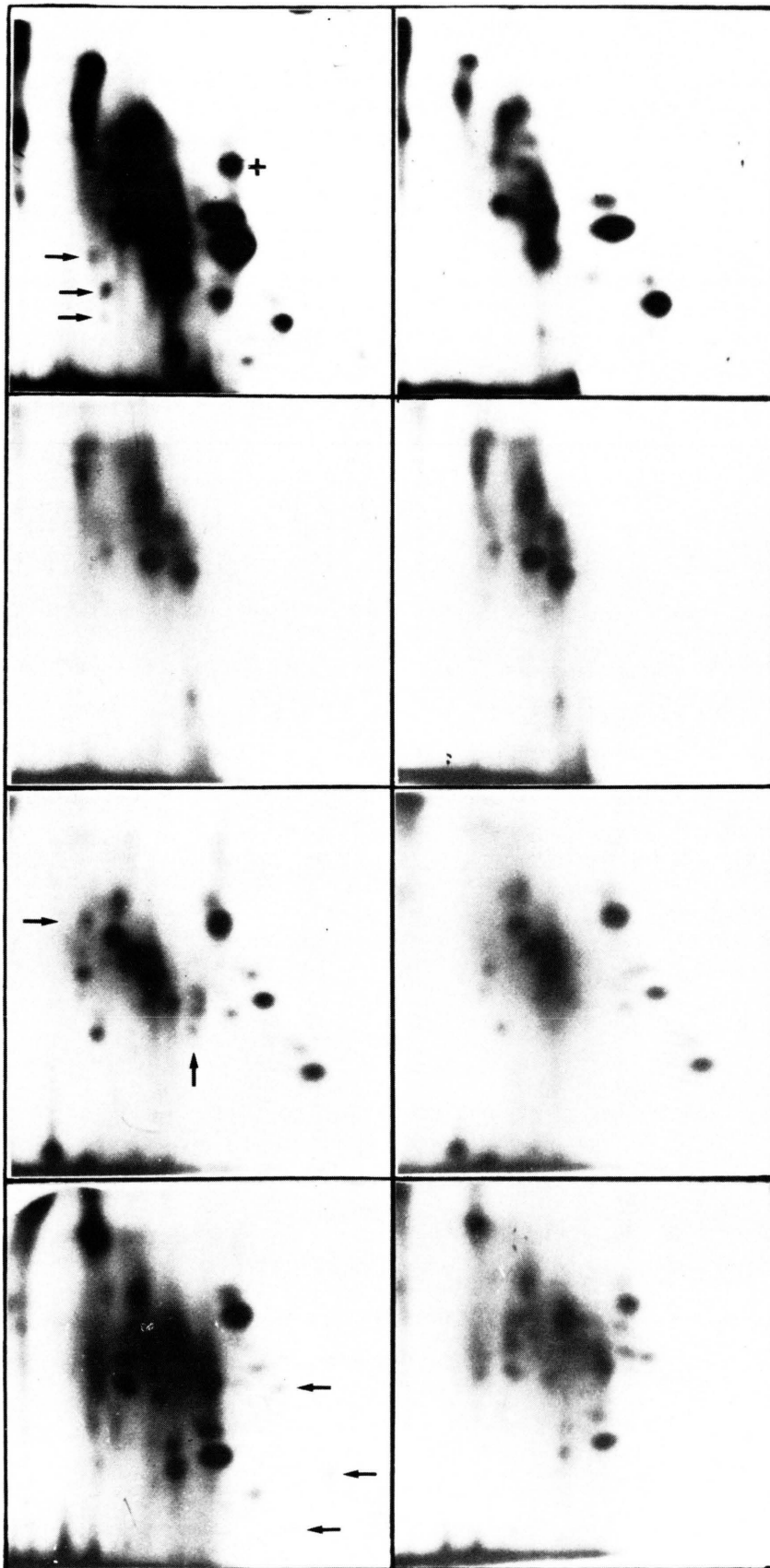
The tryptic peptide analyses of viral glycoproteins point to extensive strain specific variability in their

primary structure even in the group of closely related murine type-C viruses. However the nature of the association between glycoproteins and membrane polypeptides appears to be highly conserved in mammalian type-C viruses. On the intact viral envelope SH-groups of the glycoprotein and the membrane protein are positioned very close to each other. Only a fraction of them is oxidised to form

Fig. 2. Tryptic peptide maps of the 85 kd glycoprotein in rosette preparations from mammalian type-C viruses. Protein bands stained after PAGE with Coomassie blue were cut out and processed according to Elder *et al.* [18]. Electrophoresis of iodinated oligopeptides was performed from left to right, chromatography from bottom to top. Arrows indicate minor spots, the cross a major spot specific for gp85.

gp 85

gp 71



FLV

MoLV

B-MuX

FeLV

disulfide bridges. This particular molecular arrangement of the viral surface knob of mammalian type-C viruses is in some respect reminiscent of the structure of the influenza virus hemagglutinin [19] and may likewise be of functional significance.

We have introduced a relatively simple and efficient isolation procedure for envelope polypeptides of various mammalian type-C viruses. Preliminary results indicate that, in principle, this technique is also applicable to the preparation of glycoprotein complexes of avian type-C and murine mammary tumor viruses. At least glycoproteins of some type-C

viruses should now be available in quantities facilitating detailed structural analyses as well as immunological studies.

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- [1] J. Schneider, H. Falk, and G. Hunsmann, *J. Virol.* **33**, 597 (1980).
- [2] G. Hunsmann, V. Moennig, L. Pister, E. Seifert, and W. Schäfer, *Virology* **62**, 307 (1974).
- [3] M. E. Lostrom, M. R. Stone, M. Tam, W. N. Burnette, A. Pinter, and R. C. Nowinski, *Virology* **98**, 336 (1979).
- [4] G. Hunsmann, V. Moennig, and W. Schäfer, *Virology* **66**, 327 (1975).
- [5] W. Schäfer, H. Schwarz, H.-J. Thiel, E. Wecker, and D. P. Bolognesi, *Virology* **75**, 401 (1976).
- [6] C. Moroni and G. Schumann, *Nature* **296**, 600 (1977).
- [7] E. Wecker, A. Schimpl, and T. Hünig, *Nature* **296**, 599 (1977).
- [8] J. H. Elder, F. C. Jensen, M. L. Bryant, and R. A. Lerner, *Nature* **267**, 23 (1977).
- [9] P. J. Fischinger, A. E. Frankel, J. H. Elder, R. A. Lerner, J. N. Ihle, and D. P. Bolognesi, *Virology* **90**, 241 (1978).
- [10] J. Schneider, H. Schwarz, and G. Hunsmann, *J. Virol.* **29**, 624 (1979).
- [11] E. Weiland, M. Mussgay, and F. Weiland, *J. Exp. Med.* **148**, 408 (1978).
- [12] H. Frank, H. Schwarz, T. Graf, and W. Schäfer, *Z. Naturforsch.* **33 c**, 124 (1978).
- [13] A. Pinter and E. Fleissner, *J. Virol.* **30**, 157 (1979).
- [14] J. Schneider and G. Hunsmann, *Int. J. Cancer* **22**, 204 (1978).
- [15] A. Pinter, J. Liemann-Hurwitz, and E. Fleissner, *Virology* **91**, 345 (1978).
- [16] P. J. Robinson, G. Hunsmann, J. Schneider, and V. Schirmacher, *J. Virol.* **36**, 291 (1981).
- [17] G. Hunsmann, J. Schneider, and E. Wecker, *Virology* (1981) submitted.
- [18] J. H. Elder, R. A. Pickett, J. Hampton, and R. A. Lerner, *J. Biol. Chem.* **252**, 6510 (1977).
- [19] F.-X. Bosch, M. Orlich, H.-D. Klenk, and R. Rott, *Virology* **95**, 197 (1979).

Erratum

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