JOURNAL OF VIROLOGY, Apr. 1982, p. 352–355
0022-538X/82/040352-04$02.00/0

Vol. 42, No. 1

Glycoproteins of Friend Murine Leukemia Virus: Separation and NH_2-Terminal Amino Acid Sequences of gp69 and gp71

DIETMAR LINDER,[1] STEPHAN STIRM,[1] JOSEPH SCHNEIDER,[2] GERHARD HUNSMANN,[2] GARY SMYTHERS,[3] AND STEPHEN OROSZLAN[3]*

Biochemisches Institut am Klinikum der Universität, D-6300 Giessen, West Germany[1]; Forschergruppe Tumorimmunologie am Institut für Immunbiologie der Universität, D-7800 Freiburg, West Germany[2]; and Biological Carcinogenesis Program, Frederick Cancer Research Facility, National Cancer Institute, Frederick, Maryland 21701[3]

Received 24 September 1981/Accepted 27 November 1981

The NH_2-terminal amino acid sequences (initial 23 residues) of Friend murine leukemia virus gp71 and gp69 were determined and found to be different but highly related. Friend murine leukemia virus gp71 differed from Rauscher murine leukemia virus gp70 in only one position. Friend murine leukemia virus gp69 showed ~41% homology to these glycoproteins but lacked the glycosylation site (sequon) occurring at position 12 in Rauscher murine leukemia virus gp70.

During our studies (2, 13, 14) on the protein and carbohydrate chemistry of glycoprotein from Friend murine leukemia virus (F-MuLV) gp70 as produced by Eveline cells (15), we were confronted with the problem of heterogeneity of this viral product: F-MuLV gp70, as well as other MuLV glycoproteins, can be separated into isoglycoproteins which differ by charge or by size (i.e., gp69 and gp71) or both (1, 5, 8, 13). The charge heterogeneity could be attributed in part to differences in sialylation (8), but the degree of heterology between the gp69 and gp71 size classes remains unclear. Peptide mapping and serological comparison indicate a close relationship, e.g., a difference in oligosaccharide substitution or by a short deletion only (1, 5, 8, 13), whereas the finding that gp69 and gp71 are encoded by separate viral genomes (8) calls for an investigation of possible differences in amino acid sequence. In this communication, we report the NH_2-terminal amino acid sequences for both gp71 and gp69 of F-MuLV.

F-MuLV was propagated in Eveline cells and harvested by ultracentrifugation (15). gp69-gp71 was solubilized from the viral particles (130 mg of protein) by freezing and thawing in the presence of sodium chloride, as described previously (7), but employing 0.5 M NaCl in 0.01 M Tris-hydrochloride buffer (pH 7.4). After pelleting of the viral residues (7), 50 mg of solubilized protein was obtained and subjected to ion-exchange chromatography on phosphocellulose. The peak eluting between 0.1 and 0.2 M NaCl contained 5 mg of protein as determined by amino acid analysis. It consisted of a mixture of gp71 and gp69, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1, lane b). gp71 and gp69 were then separated by preparative SDS-PAGE (6, 13) (Fig. 1, lanes c and d), employing the detection and elution techniques of Hager and Burgess (3). The final yields of the two glycoproteins were 2.3 (gp71) and 0.8 mg (gp69) of protein.

For NH_2-terminal amino acid sequence analysis, the purified glycoproteins, as well as the mixture, were first subjected to high-performance liquid chromatography. The samples were dialyzed against 0.02% aqueous SDS, acidified to pH 2 with 10% aqueous trifluoroacetic acid, and adsorbed to a μBondapak C_{18} column (0.42 by 30 cm) of a Waters high-performance liquid chromatographic system. Elution was carried out with a linear gradient of acetonitrile (0 to 60%) in 0.05% aqueous trifluoroacetic acid, recording the absorbance at 206 nm. Light-absorbing fractions were analyzed for gp71 and gp69 by SDS-PAGE. High-performance liquid chromatography under the conditions used did not resolve gp71 and gp69 but removed contaminants interfering with sequencing. The glycoproteins were recovered by lyophilization and subjected to semiautomated Edman degradation in a Beckman model 890C sequenator, using polybrene as a carrier (16) and running the 0.1 M Quadrol protein program with extended drying times (10). The proteins were double-coupled in the first cycle; single coupling and single cleavage were employed thereafter. The thiazolinone derivatives were converted with 1 M HCl (9), and the phenylthiohydantoin (PTH) amino acids were identified by high-performance liquid chromatography (4; Table 1). As seen, a single amino acid residue was identified for gp71 throughout 23 cycles (except cycle 12 where no PTH amino acid was detected). The same amino acids were also found in at least 21 respective cycles by

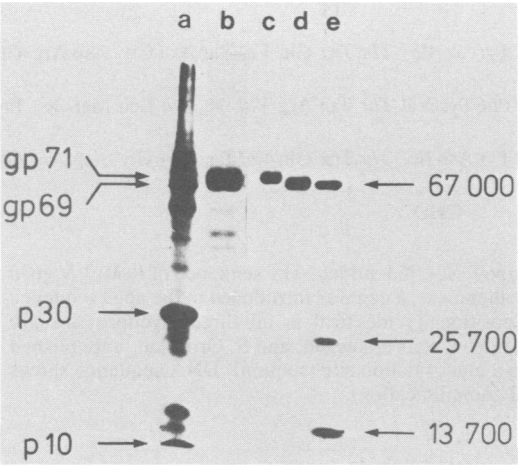

FIG. 1. Separation of F-MuLV gp69 and gp71 by SDS-PAGE (6). Samples (0.5 to 1.0 mg of protein per ml) were dissolved by heating (3 min at 100°C) in 0.1 M Tris-hydrochloride buffer (pH 6.8) containing 1% SDS and 1% 2-mercaptoethanol. For preparative separations, 1.5-ml portions were run on 3-mm 10% polyacrylamide gels and stained with KCl (3). The gp69 and gp71 bands were cut, crushed, and extracted with 0.05% aqueous SDS (total yield, about 2.3 and 0.8 mg of protein of gp71 and gp69, respectively). For analytical purposes, 20-μl portions were used, and Coomassie blue was used for staining. Lane a, Complete F-MuLV; lane b, peak fraction containing gp69/gp71 after phosphocellulose chromatography; lanes c and d, purified gp71 and gp69 after preparative SDS-PAGE; lane e, protein standards (bovine serum albumin, molecular weight, 67,000; bovine chymotrypsinogen A, molecular weight, 25,700; bovine RNase, molecular weight, 13,700).

analyzing the mixture (gp69/gp71). The mixture (gp69/gp71) and even SDS-PAGE-purified gp69 yielded at several cycles two identifiable PTH amino acids. Thus, these data indicate that SDS-PAGE-purified gp71 was completely free of gp69, whereas gp69 fraction still contained a minor amount of (subsialylated?) gp71. On the basis of the quantitative data of Table 1, the NH_2-terminal amino acid sequences of F-MuLV gp71 and gp69 were deduced (Fig. 2) and aligned with each other and with the NH_2-terminal sequence of Rauscher (R) MuLV gp70 (11).

It can be seen that the NH_2 terminus of F-MuLV gp71, which was recently verified by nucleic acid sequencing (W. Koch and R. Friedrich, personal communication), is very similar to that of R-MuLV gp70 (11), with only a single amino acid exchange at position 16 (Glu→Gln),

TABLE 1. NH_2-terminal sequence analysis of F-MuLV glycoproteins

Cycle	Amino acids identified			Cycle	Amino acids identified		
	gp71/gp69	gp71	gp69		gp71/gp69	gp71	gp69
1	Ala (2.3)[a] Val (2.2)	Ala (1.1)	Ala (0.3) Val (0.7)	13	Ile (1.0) Thr (0.5)	Ile (0.2)	Ile (0.1) Thr (0.2)
2	Ala (2.0)	Ala (0.5)	Ala (0.2)	14	Thr (0.6) Trp (0.3)	Thr (0.1)	Trp (0.1)
3	Pro (1.2)	Pro (0.5)	Pro (0.4)	15	Trp (0.2)	Trp (0.1)	Arg (0.2)
4	Gly (1.2)	Gly (0.2)	—[b]	16	Glu (0.3) Val (0.9)	Glu (0.2)	Glu (0.2) Val (0.3)
5	Ser (0.2) Thr (0.3)	Ser (0.1)	Ser (0.1) Thr (0.1)	17	Val (1.1) Thr (0.5)	Val (0.2)	—
6	Thr (0.3) Ser (0.1)	Ser (0.1)	Pro (0.4)[c]	18	Thr (0.5) Asn (0.2)	Thr (0.1)	Thr (0.2) Asn (0.2)
7	Pro (0.9)	Pro (0.5)	Pro (0.4)	19	Asn (0.2) Leu (0.7)	Asn (0.2)	Leu (0.2)
8	His (0.7) Gln (0.4)	His (0.2)	Gln (0.3)	20	Gly (0.4) Met (0.3)	Gly (0.1)	Met (0.1)
9	Gln (0.5) Val (1.2)	Gln (0.3)	Val (0.4)	21	Asp (0.2) Thr (0.5)	Asp (0.2)	—
10	Val (2.0) Phe (0.9)	Val (0.2)	Phe (0.3)	22	—	Arg (0.1)	Pro (0.2)
11	Tyr (1.2)	Tyr (0.3)	Tyr (0.3)	23	Glu (0.4) Gln	Glu (0.2)	Glu (0.1)
12	Val (1.2)	—	Val (0.4)				

[a] The numbers in parentheses indicate nanomoles of amino acids recovered at each cycle. Data are not corrected for background.

[b] —, None.

[c] The identification of Pro at cycle 6 of gp69 is unambiguous, but Pro could not be quantified at cycle 6 of gp71/gp69 because of the malfunction of the high-performance liquid chromatographic system.

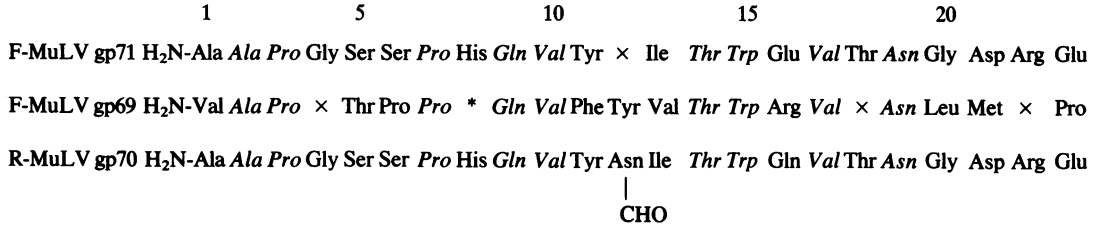

FIG. 2. NH_2-terminal sequences of F-MuLV gp71 and gp69. X, Unidentified. The sequence of R-MuLV gp70 is taken from Oroszlan and Gilden (11). In position 8 of the alignment, a gap was introduced in the gp69 sequence to obtain maximal homology. Amino acids which are positionally identical in all three glycoproteins are italicized. Asn_{12} of R-MuLV gp70 is glycosylated (L. Henderson, R. Versteegen, and S. Oroszlan, unpublished data). The same position in F-MuLV gp71 is probably also a glycosylation site (sequon); DNA sequence shows the presence of Asn (W. Koch and R. Friedrich, personal communication).

but differs substantially from that of gp69. In spite of differences observed, the NH_2-terminal amino acid sequence of F-MuLV gp69 showed a high degree (41%) of homology to both R-MuLV gp70 and F-MuLV gp71. Out of 22 residues, 9 were positionally identical. Most of these amino acids are also conserved in feline leukemia virus gp70 (12). Since the NH_2-terminal sequence of gp69 does not occur more proximally within gp71, it is clear that the size difference between gp71 and gp69 cannot be due to the deletion of an NH_2-terminal 2,000-dalton fragment. It may rather be related to the lack of an NH_2-terminal glycosylation site (sequon) in gp69 which occurs at position 12 in R-MuLV gp70 (12; L. E. Henderson, R. J. Versteegen, and S. Oroszlan, unpublished data). The same position in F-MuLV gp71 is a potential glycosylation site since DNA sequence data show the presence of Asn (W. Koch and R. Friedrich, personal communication).

Our results are in agreement with the proposal of Murray and Kabat (8) that glycoproteins gp69 and gp71, found in many MuLV preparations, may exhibit a considerable degree of difference in amino acid sequence. Of the possible explanations for the occurrence of gp69 besides gp71, simple contamination of the latter with unrelated cellular or viral material is ruled out by the high degree of sequence homology, as reported here and as previously suggested by tryptic fingerprinting of the two components (1, 5, 8, 14). It is most likely that F-MuLV gp71 and gp69 are encoded by different but related viral genes. Moreover, it is possible that gp69 is a glycoprotein which results from the recombination of ecotropic and endogenous xenotropic MuLV. This is especially intriguing in view of the presence of mink cell focus-forming recombinant virus in the Friend virus complex (17). Similar recombinant virus may be produced by Eveline cells.

This work was supported by Deutsche Forschungsgemeinschaft (SFB 47 and Hu 244/6) and Fonds der Chemischen Industrie (341) and by the National Cancer Institute under contract no. 75380 with Litton Bionetics, Inc.

LITERATURE CITED

1. **Elder, J. H., F. C. Jensen, M. L. Bryant, and R. A. Lerner.** 1977. Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: virion associated and differentiation antigens encoded by a multi-gene family. Nature (London) **267**:23–28.
2. **Geyer, R., G. Hunsmann, and S. Stirm.** 1980. Glycopeptides from Friend leukemia virus glycoprotein 71 (gp71). Hoppe Seyler's Z. Physiol. Chem. **361**:249–250.
3. **Hager, D. E., and R. R. Burgess.** 1980. Elutions of proteins from sodium dodecyl sulfate-polyacrylamide gels, removal of sodium dodecyl sulfate, and renaturation of enzymatic activity: results with sigma subunit of *Escherichia coli* RNA polymerase, wheat germ DNA topoisomerase, and other enzymes. Anal. Biochem. **109**:76–86.
4. **Henderson, L. E., T. D. Copeland, and S. Oroszlan.** 1980. Separation of amino acid phenylthiohydantoins by high-performance liquid chromatography on phenylalkyl support. Anal. Biochem. **102**:1–7.
5. **Krantz, M. J., M. Strand, and J. T. August.** 1977. Biochemical and immunological characterization of the major envelope glycoprotein gp69/71 and degradation fragments from Rauscher leukemia virus. J. Virol. **22**:804–815.
6. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
7. **Marquardt, H., R. V. Gilden, and S. Oroszlan.** 1977. Envelope glycoproteins of Rauscher murine leukemia virus: isolation and chemical characterization. Biochemistry **16**:710–717.
8. **Murray, M. J., and D. Kabat.** 1979. Genetic and sialylation sources of heterogeneity of the murine leukemia virus glycoproteins gp69/gp71. J. Biol. Chem. **254**:1340–1348.
9. **Oroszlan, S., T. D. Copeland, M. R. Summer, G. Smythers, and R. V. Gilden.** 1975. Amino acid sequence homology of mammalian type C RNA virus major internal proteins. J. Biol. Chem. **250**:6232–6239.
10. **Oroszlan, S., T. D. Copeland, L. E. Henderson, J. R. Stephenson, and R. V. Gilden.** 1979. Amino-terminal sequence of bovine leukemia virus major internal protein: homology with mammalian type C virus p30 structural proteins. Proc. Natl. Acad. Sci. U.S.A. **76**:2996–3000.
11. **Oroszlan, S., and R. V. Gilden.** 1980. Primary structure analysis of retrovirus glycoproteins, p. 299–344. *In* J. R. Stephenson (ed.), Molecular biology of RNA tumor viruses. Academic Press, Inc., New York.
12. **Oroszlan, S., L. E. Henderson, T. D. Copeland, A. M.**

Schultz, and E. M. Rabin. 1980. Processing and structure of murine leukemia virus *gag* and *env* gene-encoded polyproteins, p. 219–232. *In* G. Koch and D. Richter (ed.), Biosynthesis, modification, and processing of cellular and viral polyproteins. Academic Press, Inc., New York.

13. **Schneider, J., H. Falk, and G. Hunsmann.** 1980. Envelope polypeptides of Friend leukemia virus: purification and structural analysis. J. Virol. **33**:597–605.
14. **Schneider, J., H. Schwarz, and G. Hunsmann.** 1979. Rosettes from Friend leukemia virus envelope: preparation and physiochemical and partial biological characterization. J. Virol. **29**:624–632.
15. **Seifert, E., M. Claviez, H. Frank, G. Hunsmann, H. Schwarz, and W. Schafer.** 1975. Properties of mouse leukemia viruses. XII. Production of substantial amounts of Friend leukemia virus by a suspension tissue culture line (Eveline suspension cells). Z. Naturforsch. **30**:698–700.
16. **Tarr, G. E., J. F. Beecher, M. Belland, and D. J. McKean.** 1978. Polyquaternary amines prevent peptide loss from sequenators. Anal. Biochem. **84**:622–627.
17. **Troxler, D. H., S. K. Ruscetti, and E. M. Scolnick.** 1980. The molecular biology of Friend erythroleukemia virus. Biochim. Biophys. Acta **605**:305–325.