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STRUCTURAL POLYPEPTIDE COMPOSITION OF A MURINE MYELOMA (MOPC-315) TYPE C RETROVIRUS

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SUMMARY

The polypeptide composition of an endogenous NB-tropic type C retrovirus of murine myeloma MOPC-315 cells was analyzed by agarose gel chromatography in 6 M guanidine hydrochloride and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Seven polypeptides were reproducibly resolved: five with estimated molecular weights of 110,000, 70,000, 45,000, 15,000 and 10,000 daltons, and two with molecular weights of 30,000 daltons. The 110,000-, 70,000- and 45,000-dalton polypeptides were found to be glycosylated, and of these the 70,000-dalton protein was the major glycoprotein, while the 45,000-dalton protein was minor and poorly glycosylated. The two 30,000-dalton polypeptides of MOPC-315 virus which were found to be identical with regard to their peptide profile share antigenic determinants with the Mo-MuLV-derived 30,000 protein but differ from it in their peptide composition. The findings of two 30,000-dalton proteins in an additional member of the myeloma viruses coupled with the presence of unusual high molecular weight glycoproteins, provides further evidence in support of the notion that the myeloma type C viruses comprise a distinct group within the murine leukemia viruses.

INTRODUCTION

The type C virus secreted by the murine myeloma MOPC-315 possesses the typical features of retroviruses, namely, a density of 1.16 g/ml in sucrose gradients, a high molecular weight RNA of 60-70S and an RNA dependent DNA polymerase (1). Recently we have demonstrated that this virus is an endogenous virus of the BALB/c mouse (2) whose genetic information had been activated and expressed into mature viral particles within the mouse myeloma cells (3, 4).

Because a substantial number of the myeloma-associated viruses display some unique biochemical, biological and immunological characteristics (5) which differentiate them from the mouse leukemia group of viruses, it has been

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suggested (6) that they comprise a different subgroup within the murine oncornaviruses. To the distinguishing features one could add the unique structure of the RNA dependent DNA polymerase associated with the murine myeloma MOPC-315 virus, which demonstrates an unusually low molecular weight of 56,000 daltons and consists of two subunits of 28,000 and 26,500 daltons (7). Also, analysis of the structural polypeptides, performed independently by two research teams (5, 8) on type C particles released by three different murine myelomas, has revealed a polypeptide pattern distinct from that in the prototype murine leukemia virus, i.e., they contained high molecular weight glycosylated proteins (5, 8), a 45,000-dalton protein (8) and, surprisingly, two 30,000-dalton proteins (5, 9). This exceptional finding of the two 30,000-dalton proteins and their possible relation to the NB-tropism of the myeloma viruses (5), prompted us to conduct a careful analysis of the protein composition of the MOPC-315 virus - an additional NB-tropic member of the myeloma viruses. The present paper: a) confirms that unusually high molecular weight glycoproteins are associated with myeloma viruses; b) shows that the 45,000-dalton protein of these particles is glycosylated; c) demonstrates the presence of two 30,000-dalton proteins identical in terms of their peptide patterns. These two polypeptides, though antigenically related to the 30,000-dalton protein of Moloney murine leukemia virus (Mo-MuLV), differ from it in their peptide composition.

MATERIALS AND METHODS

Viruses and purification procedure. The myeloma viral particles were obtained and purified as previously described (10). Culture fluids of NIH/3T3-C1 1 cells (Dulbecco's modification of Eagle's medium (GIBCO) containing 10% calf serum) chronically infected with Mo-MuLV were the source of this virus. Virus particles were concentrated and purified by sucrose density centrifugation as detailed previously (3). The B77 avian sarcoma virus (B77 ASV) was grown and purified as described earlier (11).

Preparation of radioactive particles. Internal labelling of virions was performed by incubation of cells with either 10 μCi/ml ^{14}C-amino acid mixture (54 mCi/mmole, Amersham, England) or ^{14}C-glucosamine (59 mCi/mmole, Amersham, England).

External labelling of MOPC-315 viral proteins with ^{125}I was performed employing the chloramine T technique (12).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide tube gels (7.5%) and slab gels (12%) with 5% stacking gels, both containing 0.1% sodium

dodecyl sulfate (SDS), were prepared by using the discontinuous buffer system of Laemmli (13). Purified virus particles were dissociated and electrophoresed as described previously (11). The unlabelled viral proteins were electrophoresed on slab gels and analyzed after staining with Coomassie brilliant blue. Labelled proteins were electrophoresed on tube gels, cut into 1 mm slices, transferred into scintillation vials, and incubated for 18 h at 60°C with 0.2 ml of 30% H_2O_2 to release radioactive proteins prior to counting in 2 ml Insta-Gel (Packard) scintillation solvent.

Gel filtration in 6 M guanidine hydrochloride (GuHCl). ^{125}I-labelled virus particles were dissociated and chromatographed on a Sepharose 4B column in the presence of 6 M GuHCl as described by Green and Bolognesi (14). The column dimensions were 100 x 1.5 cm and the flow rate was 1 ml/h. Fractions of 1 ml each were collected and analyzed for their labelled proteins. The column was calibrated by protein markers - ^{125}I-immunoglobulin G from rabbit serum, bovine serum albumin, and beef heart cytochrome-c.

Immunoprecipitation of the ^{125}I-labelled proteins. Immunoprecipitation of viral proteins with goat anti Mo-MuLV p30 serum followed by pig anti goat IgG, was performed as detailed elsewhere (15).

Peptide mapping. ^{125}I-labelled viral proteins were electrophoresed on a 12.5% SDS-polyacrylamide preparative slab gel, and the p30 polypeptides were eluted from the dried gel bands (15). The peptide mapping was carried out by a limited proteolysis of the eluted polypeptides using Staphylococcus aureus V8 protease (Miles Laboratories) as described in detail earlier (15).

RESULTS AND DISCUSSION

The polypeptide composition of the MOPC-315 viral particles was first analyzed chromatographically. Detergent-solubilized MOPC-315 virus was iodinated and applied to agarose gel chromatography in 6 M GuHCl. Results are demonstrated in Fig. 1. The protein nomenclature is based on the recommendations of August et al. (16). As can be seen, the profile of MOPC-315 viral polypeptides determined by agarose-GuHCl chromatography, is essentially as described for other murine leukemia viruses (16-18): a major 30,000-dalton polypeptide (p30), with other low-molecular-weight polypeptides having molecular weights of 15,000, 12,000 and 10,000 daltons (p15, p12 and p10), as well as two high-molecular-weight proteins of 73,000 and 41,000 daltons (gp69/71 and gp45).

Because determination of molecular weights by gel filtration in agarose-GuHCl is not accurate for polypeptides larger than 30,000 daltons (16) and since this procedure which entails high denaturing conditions renders some proteins, particularly glycoproteins, unstable (19-21), the MOPC viral proteins were also analyzed by SDS-polyacrylamide gel electrophoresis. To this end, cells were grown in medium containing ^{14}C-labelled amino acids, viral particles were puri-

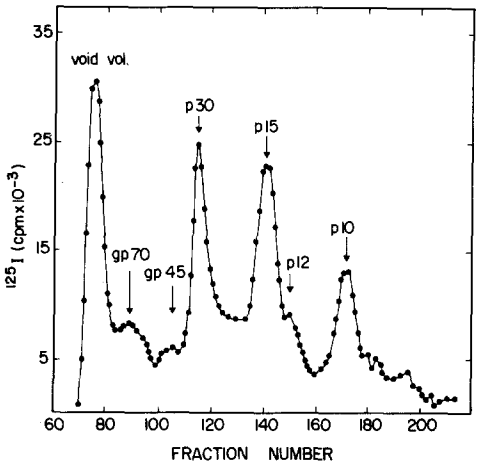

Fig. 1: Analysis of ^{125}I-labelled MOPC-315 virion polypeptides by agarose gel chromatography in 6 M GuHCl. Standard proteins for molecular weight determinations were as detailed in Materials and Methods.

fied and their proteins were electrophoresed on 7.5% polyacrylamide-SDS gels. As illustrated in Fig. 2, SDS-PAGE cylindrical tube gel analysis yielded basically a profile of polypeptides similar to that obtained by the gel filtration in agarose-GuHCl: a major viral polypeptide with a molecular weight of 30,000 daltons and the other polypeptides with molecular weights of 70,000, 45,000 and 10,000 daltons. Under these electrophoretic conditions, the 15,000- and 12,000-dalton polypeptides co-migrated. This procedure resolved an additional polypeptide possessing a molecular weight of 110,000 daltons.

In order to identify the MOPC-315 viral glycoproteins, viral particles obtained from culture fluids containing ^{14}C-glucosamine were purified, dissociated and their proteins analyzed as described above on SDS-PAGE. It is evident (Fig. 2) that gp69/70 protein contains the highest amount of ^{14}C-glucosamine label migrating in coincidence with ^{14}C-amino acid label, and represents the major virion glycoprotein. The 110,000-dalton polypeptide is also glycosylated. A similar high-molecular-weight glycoprotein has also been detected in MOPC-460 myeloma-associated viral particles (22). Likewise, the myeloma viral particles released by FLOPC-1 and MOPC-21 cells have been demonstrated (5) to contain a 90,000-dalton glyco-

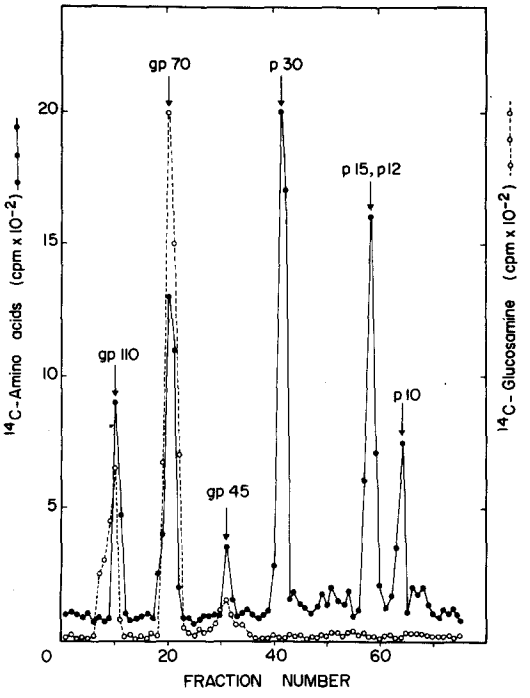

Fig. 2: Analysis of MOPC-315 viral proteins by 7.5% SDS-polyacrylamide tube gel electrophoresis. Viral particles were labelled with ^{14}C-labelled amino acids (o——o), or with ^{14}C-labelled glucosamine (o---o). Proteins used for molecular weight standards include: bovine serum albumin, immunoglobulin G and cytochrome-c.

protein. It was suggested (22) that these atypical glycoproteins may represent uncleaved precursor molecules to gp69/71 incorporated into the viral particles during assembly and maturation. In addition to these two major glycoproteins, with molecular weights of 110,000 and 70,000 daltons, a 45,000-dalton polypeptide also appeared, which contained a low level of ^{14}C-glucosamine label and therefore may constitute a minor glycoprotein of MOPC-315 virus. This polypeptide, which corresponds to the established gp45 of the murine leukemia type C particles (16), was also detected in viral particles associated with some other myelomas such as MOPC-460 (8) and SIPC-2 (9), albeit glycosylation of the latter was not observed. In fact, recent reports indicate that this gp45 may be either a poorly glycosylated form of gp71 (22) or a degradation product of gp71 (24).

Considering that the MOPC-315 virus is produced by myeloma cells, and was established as an NB tropic virus, efforts were made to determine whether this

member of the myeloma viruses, like three other NB-tropic viruses associated with mouse myelomas (5, 9), also possesses two p30 proteins. To detect the very slight difference in mobility between the two p30 polypeptides, the migration of p30 was studied, employing slab gel SDS-polyacrylamide electrophoresis so as to allow a higher resolution. The data show (Fig. 3) that in contrast to Mo-MuLV with a single band of p30 (lane B), the p30 of MOPC-315 virus migrates as doublet (lane C) - one species of p30 migrates slower than the p30 of Mo-MuLV, while the other migrates faster. In addition, it can be seen that the p15 of MOPC-315 virus migrates slightly ahead of the Mo-MuLV p15. It should be noted that similar slight differences in mobility of p15 polypeptide were also detected in other myeloma viruses, like those released by MOPC-21, FLOPC-1 (5) and SIPC-2 (9) myeloma cells.

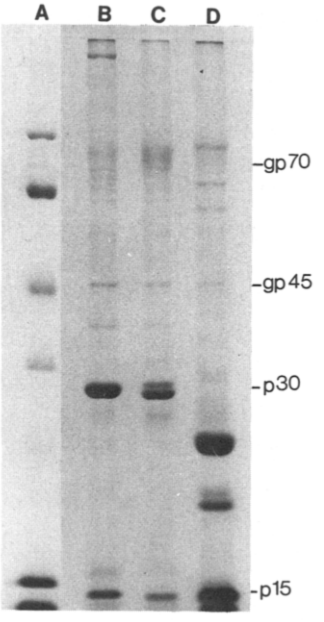

Fig. 3: Analysis of unlabelled MOPC-315 viral proteins by 12% SDS-polyacrylamide slab gel electrophoresis. (A) Standard proteins: phosphorylase A (Mr = 93,500); bovine serum albumin (Mr = 65,000); ovalbumin (Mr = 43,500); pancreatic DNase (Mr = 31,000); myoglobin (Mr = 17,000); and cytochrome-c (Mr = 13,200). (B) Mo-MuLV. (C) MOPC-315 virus. (D) B77-ASV.

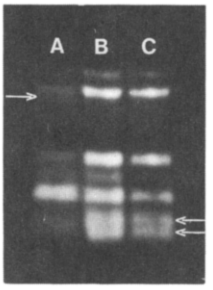

Fig. 4: The peptide pattern of the ^{125}I-labelled p30s. 7000 cpm of the p30 bands which were eluted from preparative acrylamide gel were digested with the V8 protease (at a final concentration of 500 µg/ml) for 30 min at 37°C and the peptides produced were analyzed on a 12.5-17% SDS-polyacrylamide gradient gel. (A)The products of Mo-MuLV p30. (B) MOPC-315 virus major p30 band. (C) MOPC-315 virus minor slower migrating p30 band. The gel was autoradiographed using a Kodak X-Omat R film with a Dupont Cronex Xtra life image-intensifying screen.

Experiments were carried out to gain further insight into the duality of the MOPC-315-derived p30 polypeptides. Employing immunoprecipitation assays, antiserum against Mo-MuLV p30 protein was found to precipitate both p30 polypeptides of the MOPC-315 virus (data not shown), indicating that these two p30 polypeptides are murine retroviral p30 core proteins. In spite of this immunological cross-reactivity it is evident from the profile of the peptides, generated by staphylococcal V8 protease (Fig. 4), that the MOPC-315 virus p30 polypeptides are not identical to the p30 of Mo-MuLV. The peptide bands which are found in one of the viruses' p30, and not in the other, are marked by arrows. However, this peptide mapping analysis did not detect any difference between the two p30 polypeptides of the MOPC-315 virus. It is therefore possible that the difference in their electrophoretic mobilities might be due to chemical modifications such as glycosylation or phosphorylation.

Inasmuch as the p30 protein assumedly carries the tropism determinant (25), and since it has been shown that several ecotropic viruses which differ in their N versus B tropism are distinguished also by p15 mobility (26), it is tempting to speculate that the presence of double p30 proteins as well as a unique p15 might be linked to the capacity of the MOPC-315 virus to grow efficiently in

NIH/3T3 cells as well as in BALB/c cells. It is known that several NB tropic viruses (25), including the exogenous NB-tropic Mo-MuLV (27), do not contain double p30 proteins. This feature seems to be unique to the group of myeloma viruses.

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