LOCALIZATION OF MAJOR GROUP-SPECIFIC POLYPEPTIDES IN THE AVIAN RNA TUMOR VIRUSES

Katarína Poláková, G.Russ

Cancer Research Institute and Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia

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SUMMARY. Chloramine T iodination of intact avian myeloblastosis virus revealed, that the four major group-specific polypeptides were labeled unequally, with the largest polypeptide 4 (m.w. 23,000) being preferentially iodinated, when the labeling time was limited. By contrast, in enzymatic iodination of intact virus only major virion glycoprotein and glycolipids were extensively iodinated. These observations suggest that the protein 4 surrounds other group-specific polypeptides and is itself enveloped by the bilayer of glycolipids.

The proteins and glycoproteins of avian RNA tumor viruses have been studied in a number of laboratories with respect to their number, size and antigenicity (1-4). Glycoproteins of these viruses were found to be located on the virus envelope (5-7). Four major polypeptides, exhibiting group-specific antigenicity, are located in the interior of the virus particle (8,9). In the present communication these polypeptides are designated 1 through 4 in order of increasing molecular weight, according to Bolognesi and Bauer (2). The arrangement of these components in the interior of the virus particles is not fully understood. Initial attempts to solve this problem led to isolation and characterization of the virus substructures. These studies showed that the innermost component of the virus, RNP, contained as the major

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Abbreviations: AMV - Avian myeloblastosis virus, gs - group-specific, RNP - ribonucleoprotein.

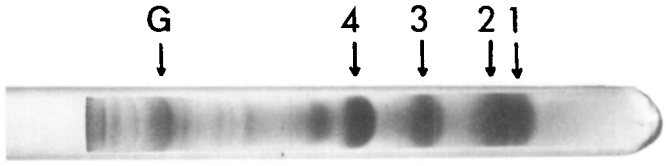

Fig.1. Intact AMV was treated with SDS and dithiotreitol, and analysed by polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with coomassie brilliant blue. Migration is from left to right. The main polypeptides are marked according to Bolognesi and Bauer (2), G is glycoprotein.

component polypeptide 2 (10-12). We have studied the localization of four major gs polypeptides in AMV by chloramine T and enzymatic iodination.

MATERIALS AND METHODS. ^{125}I (carrier free) was supplied by ROTOP, Dresden, G.D.R.. Lactoperoxidase was prepared after the method of Morrison and Hultquist (13). The BAI-A strain of AMV from the plasma of leukemic chicken was purified by differential centrifugation, followed by equilibrium centrifugation in a 5-25% Ficoll gradient. Chloramine T iodination of AMV: to a 50μl of AMV (30-50μg), 50μl of ^{125}I (0.5mCi), and 10μl of chloramine T (1 or 10μg) were added. The reagents were mixed and allowed to react for various time at 4^{o}C. Tenμl of potassium metabisulfite (2.5 or 25μg) was added to stop the reaction, followed by 10μl 0.5M NaI (14). Unreacted iodine was removed by dialysis. Enzymatic labeling was performed essentially as described by Phillips and Morrison (15). The procedures used for polyacrylamide gel electrophoresis and molecular weight estimation have been described (16). Gels containing iodinated proteins were cut into 2 mm slices with fractionator and counted in a Tesla NZQ 612 gamma counter.

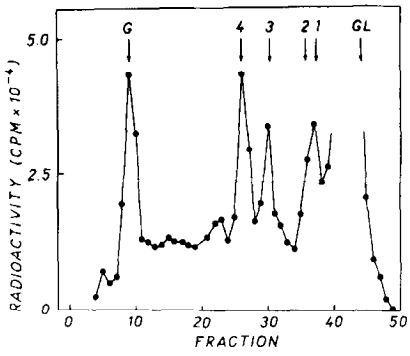

Fig.2. AMV disrupted with 1% Triton-X-100 was iodinated using chloramine T. Ten μg of chloramine T was added into a reaction mixture. The reaction was stopped after 5 min. After dialysis the virus was treated with SDS and dithiotreitol, and analysed by polyacrylamide gel electrophoresis. Migration is from left to right. GL are glycolipids.

RESULTS AND DISCUSSION. Analysis of AMV proteins by SDS polyacrylamide gel electrophoresis revealed four major bands after staining (Fig.1). These bands correspond to the major gs polypeptides of AMV (1-4). In addition to these major components a series of smaller bands were detected. The most distinct of these bands is associated with the major virion glycoprotein. Molecular weights estimates for the major proteins and the glycoprotein are given in Table 1. Slight differences in the reported size of virion proteins reported here and elsewhere (1-4) are most likely due to differences in viral sources, preparation techniques, and anomalous behaviour of glycoproteins in SDS polyacrylamide gel (16). The electrophoretic pattern of iodinated proteins was similar to the pattern of the stained polypeptides (Fig.2) except major virion glycoprotein. An apparent discrepancy between staining and labeling in this case can be explained by anomalous staining properties of the AMV glycoprotein (2). In polyacrylamide gel electrophoresis of iodinated AMV proteins a consi-

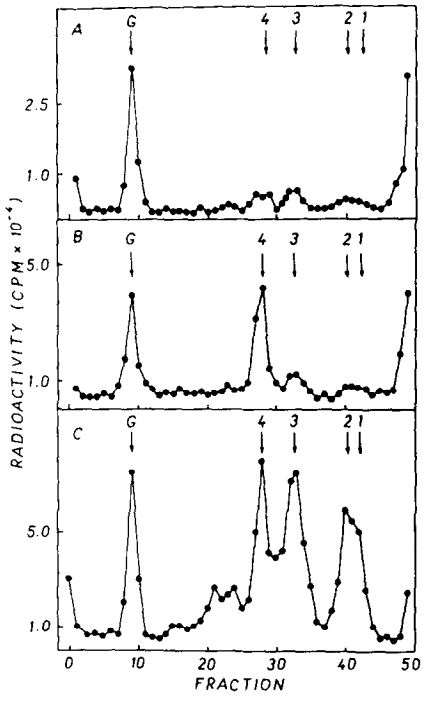

Fig.3. Intact AMV was iodinated using chloramine T. One μg (A,B) or 10μg (C) of chloramine T was added into a reaction mixture. The reaction was stopped after 1-2 sec (A), 10 sec (B), or 5 min (C). After dialysis the virus was treated with SDS and dithiotreitol, and analysed by polyacrylamide gel electrophoresis. Migration is from left to right.

derable amount of ^{125}I label migrated far down the gel to a region devoid of stained proteins. This activity corresponds to the glycolipids of AMV (11,19-21). It was estimated from 10 determinations that 65% of ^{125}I was incorporated into viral glycolipids, only remaining 35% was found in viral proteins.

Major glycoprotein and glycolipids of AMV were maximally labeled after exposure of the intact virus to the iodination agents for 1-2 sec, whereas the gs polypeptides had just begun to acquire label (Fig.3A). This result is consistent with the localization of glycoprotein on the virus enve-

lope and with four gs polypeptides placed in the virus interior (5-9). To improve separation, the polypeptides were allowed to migrate farther down the gel, therefore only the end of glycolipids peak is seen on Fig.3A-3C. With the longer exposure time unequal labeling of four gs polypeptides was demonstrated, with the largest polypeptide 4 being preferentially iodinated (Fig.3B). With the longest exposure time of intact virus to the iodination agents, similar electrophoretic pattern was obtained as after labeling of virus disrupted by Triton-X-100(Fig.2 and Fig.3C), suggesting that all reactive groups in intact virus are accesible for iodination by using chloramine T. Differential labeling of gs polypeptides was observable only with virus freshly purified on Ficoll gradient. However, even with some fresh virus stocks differential labeling of gs polypeptides was not demonstrable. The effect of storage on iodination of AMV proteins and gross morphology of this virus will be reported elsewhere (17).

The rate of labeling have been related to the position of individual polypeptides in the virions of influenza virus (14), and diplornaviruses (18). Accordingly one can suggest that the gs polypeptide 4 surrounds, probably in a form of protein membrane, other gs polypeptides in the virion of AMV. Recently Bolognesi and his coworkers suggested that this polypeptide could be the major constituent of the core shell (5). It seems very probably, that the innermost component associated with viral RNA, is gs polypeptide 2, since this basic protein was found by several authors as the major component of the inner RNP (10-12). However, we were not able to detect differences in the rate of labeling of gs polypeptides 1-3, although further time intervals were also

Table 1. Molecular weight estimates of major viral proteins.

Band no.	m.w.
1	10,000
2	12,000
3	17,000
4	23,000
G	70,000

tested. Our results taken together with the protein composition of inner RNP (10-12), strongly suggest, that the gs polypeptides 1 and 3 should be localized between the RNP and the gs polypeptide 4 membrane. However, polypeptides corresponding to our polypeptides 1 and 3 have not been detected in the virus core (5). Moreover, it was suggested that the polypeptide 1 is in fact a surface glycoprotein (5). We have not observed anomalous behaviour of gs polypeptide 1 on SDS polyacrylamide gel, although this should be expected for the glycoprotein with 5% of carbohydrate (16). In addition, polypeptide 1 was not labeled, when the intact virus was iodinated by lactoperoxidase (see bellow). We suppose that the possible contamination of polypeptide 1 by glycolipids is not completly excluded.

Labeling of intact AMV by the noninvasive enzymatic lactoperoxidase technique led to the extensive iodination of major virion glycoprotein and glycolipids (the electrophoretic pattern was very similar to the pattern given on Fig.3A). At these conditions all four gs polypeptides were very poorly iodinated. We suppose that this was due to a small population

of disrupted virion in the virus preparation. Only glycolipids and major virion glycoprotein were stained when the intact virus particles were exposed to fluorescein isothiocyanate (unpublished results). Thus, we conclude that the gs polypeptide 4 is wholly enclosed by the glycolipid bilayer.

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