Isolation of the Major Glycoprotein (gp70) of Simian Sarcoma Virus (SSV-1/SSAV-1) in Preparative Quantities


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Glycoprotein, Simian Sarcoma Virus, Isolation, Immuno-Adsorbent

The major glycoprotein (gp70) of simian sarcoma virus is present in “soluble” form in the medium of virus-producing suspension cultures. It could be isolated from the supernatant of such cultures in substantial amounts by an immuno-adsorbent technique. Some of its gel-electrophoretic and serological properties are described.

The major surface glycoprotein (gp71) of murine Friend leukemia virus (FLV), with an apparent molecular weight of 71000 d, is responsible for many of the biological characteristics of the virus (for review see ref. 1). Among other properties, it is able to immunize against an infection and to induce neutralizing as well as cytotoxic antibodies. Potent gp71 antisera, which were prepared by immunizing rabbits or goats with milligram amounts of isolated gp71, showed type-specific as well as interspecies reactivity. With such antisera it was possible to treat effectively not only Friend leukemia disease but also spontaneous leukemia in AKR mice and solid tumors induced in kittens by feline sarcoma virus. The latter effect is probably due to the interspecies activity of the serum.

In addition to gp71 Rauscher murine leukemia virus (RLV) contains a glycoprotein with an apparent molecular weight of 69000 d which seems to be a degradation product of gp71.

A similar major glycoprotein(s) was shown to be present in simian sarcoma virus type 1 (SSV-1/SSAV-1). However, thus far it has not been isolated in amounts large enough for further study or for producing potent, broadly reacting antibodies. Recent reports claiming that agents related to SSV play a role in human neoplasia provoked our interest in studying its structural components, especially its major glycoprotein(s). In this preliminary report we describe an isolation procedure for the major SSV glycoprotein(s), its purification in substantial amounts, and some of its properties.

A suspension culture of SSV-producing mar­moset cells (HF-SSV/Jü), derived from cultures developed by Wolfe et al., was used for these studies. The antisera employed are listed in Table I. The highly virus-specific, goat anti-SSV serum (g-SSV-serum) was prepared by the autologous immunization of goats, and g-SSV-serum contains antibodies to the viral surface glycoprotein(s) and that this could represent line 1 of the Ouchterlony test. Neither in CF nor in Ouchterlony tests did g-SSV-serum react with isolated p30 or p10 of SSV, with fetal calf serum or with extracts of normal marmoset cells.

Our earlier studies showed that the major glycoprotein of murine leukemia virus is easily released from the viral and host cell surface and that it is present in substantial amounts in soluble form in the medium. The same appears to be true for SSV.

* This virus was originally isolated from a woolly monkey and will be referred to in the following as SSV.
### Table I. Antisera.

<table>
<thead>
<tr>
<th>Designation</th>
<th>Origin</th>
<th>Prepared against</th>
<th>Reacting with SSV components</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>g-SSV-serum</td>
<td>goat</td>
<td>SSV-infected autologous goat cells</td>
<td>major glycoprotein(s), p15(E), p12(E)</td>
<td>Deinhardt et al., in preparation</td>
</tr>
<tr>
<td>SSV-p30-serum</td>
<td>rabbit</td>
<td>isolated p30 of SSV</td>
<td>p30</td>
<td>Thiel, unpublished</td>
</tr>
<tr>
<td>SSV-pl0-serum</td>
<td>rabbit</td>
<td>isolated pl0 of SSV</td>
<td>pl0</td>
<td>Thiel et al., in preparation</td>
</tr>
<tr>
<td>FLV-pl5(E)-serum</td>
<td>rabbit</td>
<td>isolated p15(E) of murine Friend leukemia virus</td>
<td>p15(E), p12(E), by interspecies reactivity</td>
<td>Schäfer et al., Deinhardt et al., in preparation</td>
</tr>
<tr>
<td>fcs-serum</td>
<td>rabbit</td>
<td>fetal calf serum</td>
<td>—</td>
<td>Hunsmann et al.</td>
</tr>
</tbody>
</table>

* a MuLV p12(E) has been shown to be biochemically and serologically related to p15(E) \(^{17}\). Respective components, which are both precipitable by FLV-p15(E)-serum, have been detected recently in SSV as well (see Deinhardt et al., in preparation).

If the virus was removed from medium of HF-SSV/Jü cultures by two cycles of ultracentrifugation at pi 10 and pi 30 \(^{19}\) respectively, about 95% of the original CF activity, as determined with g-SSV-serum, was present in the supernatant. To isolate the viral glycoprotein, the supernatant was therefore collected and passed over an immunoadsorbent column prepared with IgG from g-SSV-serum. The IgG was isolated from the serum by ammonium sulfate precipitation and subsequent DE52 chromatography, and was coupled to Sepharose 4B Cl with cyanogen bromide \(^{20}\). The adsorbed antigens were eluted with 2.5 and subsequently with 4 M MgCl\(_2\), and the eluate obtained after treatment with 4 M MgCl\(_2\) was concentrated in an Amicon ultrafiltration cell using a PM 10 membrane. When the concentrated eluate was analyzed in SDS-polyacrylamide gel electrophoresis (PAGE) \(^{21}\), a prominent band of Coomassie blue stainable material became detectable (Figure 2). This material was also stainable by the periodic acid Schiff reagent (PAS) and is therefore likely to represent glycoprotein. Two other, faster migrating Coomassie blue stainable components, possibly of fetal calf serum origin, were still present in barely detectable amounts (not recognizable in Fig. 2). The glycoprotein isolated has an apparent molecular weight of ~70000 (see location after PAGE in Fig. 2) and will be designated as gp70 of SSV. In a further purification step the minor amounts of fetal calf serum components still associated with the gp70 were removed by treatment with an appropriate immuno-adsorbent. By the procedure described we obtained ~0.5 mg of relatively pure gp70 from 41 of medium.

The purified material had a very high specific activity in CF with g-SSV-serum, with as little as 5 x 10^{-8} g protein still yielding a positive reaction. When reacted with g-SSV-serum in Ouchterlony tests it formed a single, prominent line (Fig. 1 b, c). As expected, this line was continuous with the precipitation line 1 formed by degraded total SSV (Fig. 1 b). With sera prepared against p10 and p30 of SSV, p15(E) of FLV (Fig. 1 c) and fetal calf serum (not shown), the purified material formed no detectable precipitates, even when used at a concentration of about 0.5 mg/ml. At present we are unable to decide whether our glycoprotein isolate consists of one component only or whether it contains...
minor amounts of a second component comparable to the gp69 of RLV. Experiments to clarify this point are under way.

The results presented show that gp70 of SSV can be isolated in a relatively pure form and in substantial amounts by a technically rather simple method. We hope that this will allow the production of potent antisera with capacities comparable to those of the antisera against Friend virus gp71.

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