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

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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 group and interspecies reactivity. With such antisera it was possible to treat effectively not only Friend leukemia disease [2] but also spontaneous leukemia in AKR mice [3, 4] and solid tumors induced in kittens by feline sarcoma virus [5]. 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 [6, 7].

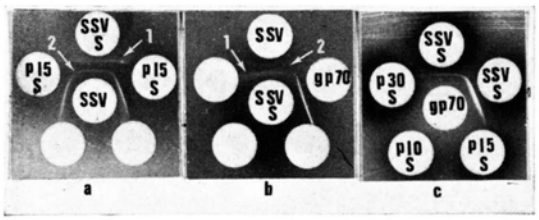

Fig. 1. Ouchterlony tests. a) Reactivities of degraded purified SSV with g-SSV-serum (SSV-S) and FLV-p15(E)-serum (p15-S). b) Reactivity of the isolated gp70 and of degraded SSV with g-SSV-serum. c) Reactivity of the isolated gp70 with g-SSV-serum and with antisera reacting specifically with SSV structural proteins (s. Table I). p30-S: SSV-p30-serum; p10-S: SSV-p10-serum; p15-S: FLV-p15(E)-serum.

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A similar major glycoprotein(s) was shown to be present in simian sarcoma virus type 1 (SSV-1/SSAV-1) *, [8, 9]. 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 [12–14] 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.* [11], 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 implantation of the goats's own cells, previously infected and transformed with SSV and grown in tissue culture medium with goat serum. g-SSV-serum neutralized SSV to a titer of about 1:4000 and had a complement fixing titer of about 1:100 when reacted with purified SSV. In Ouchterlony tests it delivered two prominent precipitation lines (Fig. 1 a) with Tween-ether degraded, purified SSV. One of these lines (line 2) was identified as to be caused by p15(E) antigen since the respective component reacted with FLV p15(E)-serum, an antiserum known to possess a high interspecies activity (Table I). Occasionally a further, very faint line was found between line 1 and 2 whose identity remains unclear. The high neutralizing capacity of the serum indicated that besides p15(E)-antibodies the 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 [18]. The same appears to be true for SSV.

* This virus was originally isolated from a woolly monkey [10, 11] and will be referred to in the following as SSV.

Table I. Antisera.

Designation	Origin	Prepared against	Reacting with SSV components	References
g-SSV-serum	goat	SSV-infected autologous goat cells	major glycoprotein(s), p15(E), p12(E) [a]	Deinhardt *et al.*, in preparation
SSV-p30-serum	rabbit	isolated p30 of SSV	p30	Thiel, unpublished
SSV-p10-serum	rabbit	isolated p10 of SSV	p10	Thiel *et al.*, in preparation
FLV-p15(E)-serum	rabbit	isolated p15(E) of murine Friend leukemia virus	p15(E), p12(E), by interspecies reactivity	Schäfer *et al.*[15], Deinhardt *et al.*, in preparation
fcs-serum	rabbit	fetal calf serum	—	Hunsmann *et al.*[16]

[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 4 l of medium.

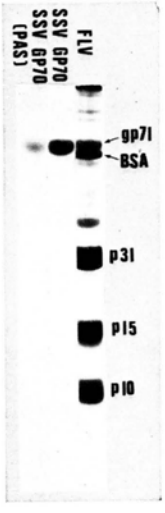

Fig. 2. SDS-polyacrylamide gel electrophoresis of isolated gp70 of SSV (SSV gp70) and of total murine Friend leukemia virus (FLV). Staining of the gels: left, PAS; middle and right, Coomassie blue. BSA = bovine serum albumin, M.W. 68000 d, contained in the virus concentrate.

The purified material had a very high specific activity in CF with g-SSV-serum, with as little as 5×10^{-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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