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Purification of simian immunodeficiency virus, SIV_{MAC251}, and of its external envelope glycoprotein, gp148☆

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Abstract

Two-phase extraction in a system composed of dextran and polyethylene glycol was used to purify simian immunodeficiency virus, SIV_{MAC251} (*32H* isolate) from 25 l of culture supernatant. The virus partitioned to the interphase with 80% recovery of *gag* peptide p27 and reverse transcriptase and an about 25% recovery of the external *env* glycoprotein, gp148.

The virus was treated with octylglycoside and its subcomponents separated. Two *gag*-p27 containing fractions were obtained; *gag*-1, which also contained reverse transcriptase and nucleopeptides, and *gag*-2, which contained the major portion of the p27. The *env* gp148 was purified by chromatography through a series of lectin columns. The prepared materials are characterized by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and immuno- and lectin blotting.

1. Introduction

In human immunodeficiency virus (HIV) and related viruses the *env* glycoproteins form protrusions from the envelope. These structures are essential for binding of the virus to the target cell and for the infection process, as discussed by Gelderblom [1]. The external *env* glycoprotein is non-covalently attached to the transmembrane glycoprotein and is easily shed into the surrounding medium. After ultracentrifugation its recovery in the virion fraction is usually poor [2,3]. This is probably due to shear forces developing during high spin. Similar labile "knob and socket" mechanisms linking the *env* proteins applies to several retroviruses [4]. Different structural changes occur on release of the external glycoprotein [5,6]. Therefore, in the purification of virus for functional studies, or for use as reference material in vaccine studies, care should be taken to preserve these membrane structures.

In the search for suitable methods to purify retroviruses we tried extraction in aqueous polymer systems [7–11]. With several of the viruses tested we found systems yielding an increased recovery of the external glycoprotein, compared to high spin ultracentrifugation [7–9]. Faced with

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the problem of purifying SIV_{MAC} and its external *env* glycoprotein, gp148, for use within an European Community (EC)-concerted research programme, European vaccine against AIDS (Programme EVA), we applied the two-phase technique to concentrate the virus [10,11]. Purification of gp148 directly from culture supernatant was recently reported [12]. In the present paper we use the virus, concentrated by two-phase extraction, as start material for purification of the glycoprotein.

2. Experimental

2.1. Virus production

Production of virus was established with the SIV_{MAC251} *32H* isolate in roller bottle cultures (500 ml/flask) of the human T-cell line *C8166*. The growth medium RPMI-1640, supplemented with 5% fetal calf serum, 2 m*M* L-glutamine, penicillin (100 IU/ml) and streptomycin (100 μg/l), in 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) buffer, pH 7.4, was used. The virus was harvested at peak production of reverse transcriptase, day 5 after infection. The flasks (roller bottles) used for culture of infected cells were left standing upright in the incubator the last night before virus harvest, to allow the cells to sediment to the bottom.

2.2. Detergents

Isotridecyl poly(ethylene glycol ether)$_n$ ($n = 7$–8) (ITDP), *n*-octylglucoside and Triton X-100 were from Boehringer Mannheim, Mannheim, Germany and Empigen BB + + (N-dodecyl-N,N-dimethylglycine) was from Calbiochem, La Jolla, CA, USA.

2.3. Monoclonal antibodies and immunochemicals

For analyses by enzyme-linked immunosorbent assay (ELISA) and immunoblot monoclonal antibodies against HIV-2 or SIV proteins were obtained from the Programme EVA (Dr. H. Holmes, NIBSC, Potters Bar, UK). The antibodies were KK33 reactive against the p27, KK7 reactive against the transmembrane glycoprotein, and KK8 and KK12 reactive against the gp148 [13]. For detection alkaline phosphatase or horseradish peroxidase conjugated goat anti-mouse IgG, blotting grade, from Bio-Rad Labs., Richmond, CA, USA, were used.

2.4. ELISA

gp148-ELISA

Polystyrene microtiter plates coated with GNA were used to bind SIV-gp148. The bound glycoprotein was quantified using a monoclonal antibody in a peroxidase-linked immunoassay according to Gilljam [12].

p27-ELISA

The antigen-capture ELISA of Thorstensson *et al.* [14] was used in combination with monoclonal antibody KK33 for detection of SIV-p27.

2.5. Lectin blot analysis

Lectin blotting was performed as earlier described [15,16]. Biotinylated lectins were from Boehringer Mannheim, Vector Labs. (Burlingame, CA, USA) and E-Y Labs. (San Mateo, CA, USA). For detection avidin and biotinylated alkaline phosphatase (ABC-AP kit) from Vector Labs. were used.

2.6. Chromatography gels and columns

Concanavalin A (Con A)-Sepharose and wheat germ agglutinin (WGA)-Sepharose were from Pharmacia, Uppsala, Sweden. These gels were packed, total volumes about 20 ml, in HR16/10 columns (Pharmacia). ProSep-GNA, the affinity column with *Galanthus nivalis* lectin (GNA, Boehringer Mannheim) coupled to pore glass beads (BioProcessing, Durham, UK) was prepared as described by Gilljam [12]. The beads were packed in an 3 × 1 cm column, total volume 2.4 ml. HiTrap-Albumin-Adsorption gel was kindly supplied by Marie Buhre, Pharmacia. It was prepared from NHS-activated HiTrap matrix

(Pharmacia) with antibodies against bovine serum albumin and packed in an 5 × 1 cm column, total volume 4 ml. Agarose-*Ricinus communis* agglutinin 120 (RCA-agarose) was from BioMakor, Rehovot, Israel.

The set of affinity columns used for preparation of *gag*-1 fraction was treated as follows: Con A-Sepharose had been stored and equilibrated against 50 m*M* ammonium acetate, 500 m*M* NaCl, 1 m*M* $MnCl_2$, 1 m*M* $CaCl_2$, pH 6.8. HiTrap-Albumin-Adsorption gel was stored in the same buffer without metal ions. Before sample application both columns were washed with 20 m*M* HEPES, pH 5.0, 0.1% Triton X-100. (The first gel was regenerated with 500 m*M* methyl mannoside in the wash buffer and the second gel with 100 m*M* glycine, pH 2.8.)

The set of affinity columns used for preparation of *gag*-2 fraction was treated as follows: Con A and HiTrap-Albumin-Adsorption gels were stored in the same buffers as used above. Prior to use they were washed with carefully deaerated 20 m*M* Hepes, pH 7.5, 0.01% octylglucoside.

2.7. Preparation of virus, two gag peptide-containing fractions (gag-1 and gag-2) and purification of the external env glycoprotein

General strategy

Infection of cell cultures and harvest at day 5 of cell-free virus containing culture medium. Extraction of virus using a two-phase system with dextran and poly(ethylene glycol) (PEG). Collection of the interphase, which contains the virus. Removal of polymers by centrifugation through a sucrose cushion to obtain the virus fraction. Treatment with octylglycoside. This results in an octylglucoside (OG) soluble fraction and an octylglucoside-insoluble fraction (OG-pellet). The former was used as source for the *gag*-2 fraction and for purification of gp148. The *gag*-1 fraction was prepared from the OG-pellet. Details are given below.

Virus purification

The virus was purified by extraction in a two-phase system with 0.24% (w/w) Dextran T500 (Pharmacia) and 7.2% (w/w) of PEG-6000 (Merck–Schuchardt, Darmstadt, Germany) ($D_{0.24}PEG_{7.2}$) as earlier described [11]. Plastic bags with attached tubing were used as extraction funnels (transfer bags "5L", or for smaller volumes blood bags; Baxter Medical, Bromma, Sweden). Polymer stock solutions were prepared to contain 10 m*M* sodium phosphate, pH 7.4 and 155 m*M* NaCl, in addition to the polymer. With the aid of a peristaltic pump the following solutions were pumped, under sterile conditions, into each 5L bag: 96 g of 10% (w/w) Dextran T500, 3330 ml cell supernatant, taken directly from culture flasks through a sterile 10-ml pipette, and 576 g of 50% (w/w) PEG-6000. The bags were then left hanging in the hood for 4 h, or overnight, during which period the system separated into a large top phase and a small bottom phase (volume ratio about 300:1). The virus accumulated at the interphase (Table 1). The bottom phase was tapped off before collecting the interphase material in a Falcon tube. The tube with the interphase was filled by addition of 8% PEG in phosphate-buffered saline (PBS), turned end over end a couple of times and spun at 1000 *g* for 10 min, in a cell centrifuge, whereby the interphase was sharpened and remainings of the bottom phase separated out. The interphase material was collected and further washed three times by addition of fresh 8% PEG-6000 in a Falcon tube. Finally the interphase material was suspended in PBS (10 m*M* sodium phosphate, pH 7.4, 155 m*M* NaCl) with 1 m*M* dithiothreitol (DTT). Polymers and soluble contaminants were removed by centrifugation through a 30% sucrose layer (over a bottom layer of 60% sucrose) at 12 000 *g* for 16 h at 4°C in a Kontron TFT 71.38 rotor. The virus fraction was collected under the 30% sucrose layer.

Solubilization of virus proteins

The virus was treated with octylglycoside, 3% final concentration, at 4°C for 60 min. It was then diluted 3-fold with 100 m*M* Tris–HCl, pH 8.5, to obtain a final octylglycoside concentration of 1% before centrifugation at 12 000 *g* for 16 h at 4°C (Kontron, rotor TFT 71.38).

Table 1
Recovery of SIV external *env* protein gp148

Fraction	Volume (ml)	Protein concentration (mg/ml)	gp148		
			Total (mg)	Recovery (%)	Purification (fold)
SIV infected cell culture medium	25 000	5	9.8	100	1
Dextran-PEG extraction					
Interphase	85	12	2.55	26	32
Bottom phase	52	–	0.005	0.1	–
Virus fraction	200	0.51	2.4	25	300
Lectin-affinity chromatography					
env gp148 fraction from GNA column	8	0.13	1.04	11	12 600

Preparation of gag-1 fraction

The OG-pellet, which contained the major portion of reverse transcriptase (RT) in addition to *gag* proteins was suspended in 10% Triton X-100 and frozen at −70°C. It was thawed and 10 m*M* Tris–HCl, pH 8.5, added to obtain a final Triton X-100 concentration of 1%, cleared by centrifugation at 100 000 *g* (Kontron, rotor TST 41.14) for 60 min. The supernatant was dialyzed, first against 20 m*M* HEPES, pH 7.5, 1 m*M* DTT, 0.5% Triton X-100, then against carefully deaerated 20 m*M* HEPES, pH 5.0, 0.1% Triton X-100.

The dialyzed material was passed over the combined Con A-Sepharose and HiTrap-Albumin-Adsorption columns. Sodium chloride was added to 25 m*M* and pH corrected to 7.5 by addition of NaOH before a final centrifugation as above. The protein content was determined relative to bovine serum albumin on trichloroacetic acid-precipitated samples. The material, *gag*-1 fraction, was diluted with 20 m*M* HEPES, 25 m*M* NaCl, pH 7.5, 0.1% Triton X-100, to a protein concentration of 1 mg/ml before sampling. The material was stored frozen at −70°C.

Preparation of gag-2 fraction

The material solubilized by octylglucoside was directly applied to similar chromatographic removal of glycoproteins and albumin as the *gag*-1 fraction by passing through a set of affinity columns. After the first passage the material was extensively dialyzed against carefully deaerated 20 m*M* HEPES, 0.01% octylglucoside, pH 7.5. The obtained opalescent material was supplemented with octylglucoside to 0.1% and centrifuged at 3000 *g* for 15 h (Sorvall, rotor SS34). It was then again passed over the regenerated affinity columns and concentrated by freeze drying to about half volume. Thawed material was dialyzed against 20 m*M* HEPES, 0.1% octylglucoside, pH 8.0, sterile filtered and diluted with the same buffer to a protein concentration of 1 mg/ml. It was stored frozen at −70°C.

Purification of external SIV env glycoprotein, gp148

The gp148 contained in the OG-soluble fraction was adsorbed on Con A-Sepharose. Before elution of the glycoprotein with 500 m*M* methyl mannoside, the column was extensively washed with 10 m*M* Tris–HCl, pH 7.5, 155 m*M* NaCl (TBS). The methyl mannoside eluate was directly applied to a WGA-Sepharose column equilibrated against TBS. This column was eluted with 100 m*M* N-acetylglucosamine (GlcNAc) in TBS and the eluate directly applied to a column with ProSep-GNA. This column was washed as shown

in Fig. 4. The SIV gp148 was eluted with 500 m*M* methyl mannoside. The purified material was sampled and stored at −70°C. During preparation the material was kept at 4–8°C.

3. Results and discussion

3.1. Two-phase extraction

There were two main reasons to apply two-phase extraction for the purification of SIV_{MAC}. First the method is convenient to use with large volumes of infectious material. Second the method has a potential for recovery of native structures [11]. In this study we concentrated SIV from a batch of 25 l by direct mix of cell supernatant and polymer stock solutions. After 4 h the phases had separated and the virus could be recovered from the interphase, 300-fold concentrated. Fig. 1 shows the presence of different virus proteins in the interphase material. SIV prepared in this way has been used for vaccine studies [17] and as source for preparation of the external *env* glycoprotein for the same purpose. In addition to the two-phase extraction we included a centrifugation step to obtain the virus free of polymers. This was done by centrifugation through a sucrose layer, overnight at 12 000 *g*. In the virus fraction the recovery of the reverse transcriptase activity and gag-p27 were about 80%. The yield of gp148 was about 25%. Loss of gp148 during re-extraction and centrifugation was minimal (Table 1), which points to the glycoprotein being associated with the virion.

3.2. Solubilization and gag fractions

In our initial studies the detergent ITDP was tried for the solubilization of the virus components. With HIV-2 this non-ionic mild detergent exclusively solubilized the external *env* glycoprotein without much leakage of *gag* proteins. With SIV_{MAC} the solubilization of gp148 was poor. We therefore used octylglucoside. This has an about 10-fold higher critical micelle concentration than ITDP. However, it has the advantage of a small micelle size ($M_r \approx 8000$), which makes it dialyzable.

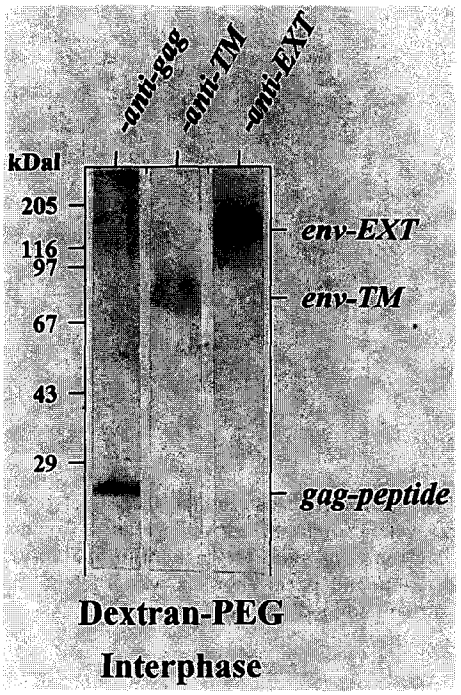

Fig. 1. SIV components recovered in the interphase in a two-phase system composed of infected cell culture supernatant, 0.24% (w/w) Dextran T500 and 7.2% (w/w) PEG-6000 as demonstrated by western blotting; the blots were probed with monoclonal antibodies against *gag*-p27 (gag-peptide), the transmembrane *env* protein (env-TM) and the *env*-gp148 (env-EXT). kDal = kilodaltons.

Treatment of the virus preparation with octylglucoside resulted in the solubilization of the major portion of gp148 and gag-p27. However, the major portion of reverse transcriptase and small *gag* peptides as well as about 5% of the p27 remained in the OG-pellet. They were solubilized by Triton X-100, depleted of N-glycosylated proteins and albumin and finally recovered as the *gag*-1 fraction. OG-soluble material was further processed to yield the *gag*-2 fraction and purified gp148.

Two fractions of *gag* protein has earlier been observed after treatment of HIV with octylglucoside and other detergents [18]. Contrary to this report we recover the major portion of the *env* proteins in the soluble fraction.

The peptide pattern of *gag*-1 and *gag*-2 fractions are shown in Fig. 2. Calculated from staining intensity of p27 and albumin bands in

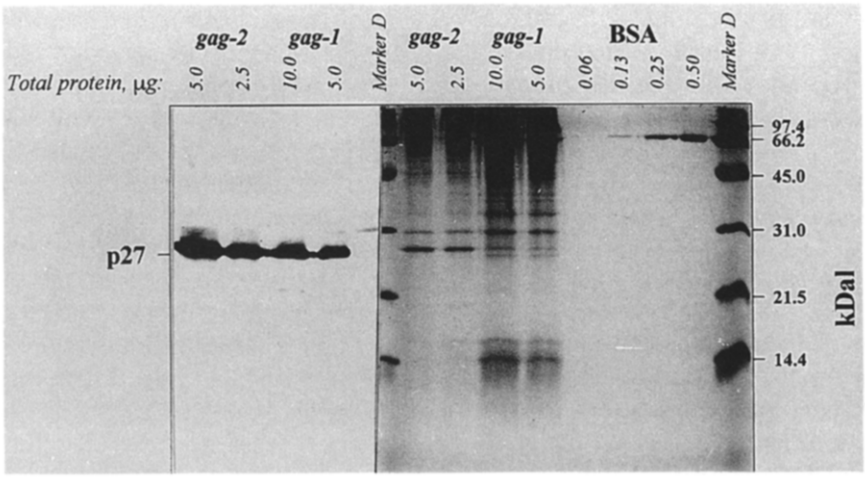

Fig. 2. SDS-PAGE and western-blot analysis of *gag*-1 and *gag*-2 fractions prepared as outlined in the Experimental section; right-hand panel shows a SDS-PAGE gel after Commassie staining. Samples of the *gag* fractions and of bovine serum albumin (BSA) were run in parallel. The amount of total protein in the applied samples and the molecular mass of marker peptides are indicated. Left-hand panel shows a western blot run with a section of the same gel. This is probed with a monoclonal anti-*gag*-protein antibody.

the Commassie stained sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) gel the p27 constitutes 2–4% of total protein in *gag*-1 fraction and 10–12% in the *gag*-2 fraction. This gives the estimated value of 30 μg/ml in the *gag*-1 and 110 μg/ml in the *gag*-2 preparation. This gives that the total yield of p27 from a 25-l culture was 840 μg (28 ml, 30 μg/ml) in the *gag*-1 fraction and 16.5 mg (150 ml, 110 μg/ml) in the *gag*-2 fraction. Both were prepared to hold a total protein concentration of 1 mg/ml.

Immunoblot of the *gag*-1 fraction (Fig. 3, not all blots are shown) with sera from HIV-2 infected humans (h-213, h-277) show, in addition to that of p27, bands at M_r 15 000, 22 000, 36 000, 44 000 and 50 000 which may represent virus peptides. The M_r 44 000 peptide binds jacalin (JAC, Fig. 3), peanut agglutinin (PNA), and the lectins from *Ricinus communis* and *Aleuria auratia* (RCA and AAA, Fig. 3), but not the lectins from *Ulex europaeus* (UEA-I) or *Lotus tetragonolobus* (Lot). The lectin binding pattern indicate an O-linked glycoconjugate with non-sialylated Galβ1→3GalNAc (Gal = galactose) core and fucose linked in such a way that it is recognized by AAA but not by UEA-I or Lot.

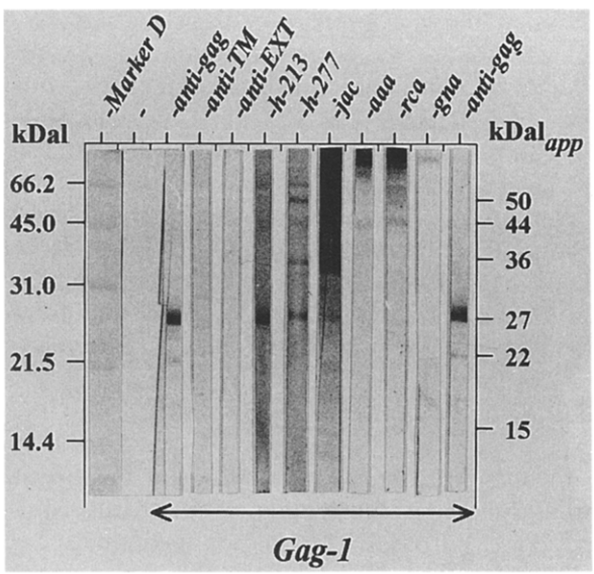

Fig. 3. Western and lectin blot analyses of the *gag*-1 fraction. The probes were monoclonal antibodies against SIV proteins (anti-*gag*, anti-TM and anti-EXT), human HIV-2 positive sera (h-213, h-277), and the lectins JAC, AAA, RCA-agl and GNA. Lectin abbreviations are explained in Table 2. Estimated molecular mass of peptides revealed by probing with the human sera are shown at the right. Marker peptides are indicated to the left.

A narrow band in the region is labeled by *Pisum sativum* agglutinin (PSA) and lentil lectin (LCA) indicative of mannose. There is no binding of GNA (Fig. 3). A summery of the lectins, the abbreviations used and reported main specificity is given in Table 2.

3.3. Purification of gp148

It may be noted that in the presence of octylglucoside the gp148 was not adsorbed to Lentil-Sepharose (Pharmacia). The binding to Con A-Sepharose was not impaired. Therefore the Con A-Sepharose was used to withdraw the glycoprotein from the virus lysate.

The lectin columns (Con A→WGA→GNA) were combined so that the eluting sugar in one system should not affect the binding to the next. The elution profile from the GNA column is shown in Fig. 4. It was found essential to carefully wash the column before elution with α-methylmannoside in order to obtain the desired purity and a sharp elution of the gp148. If the column was washed with only TBS before elution with methylmannoside a broad elution peak was obtained. If eluted with the mannoside in 1 *M* NaCl the peak was sharpened but contaminating proteins present, as shown in sample c of Fig. 5B. Small peptide contaminants were removed by washing the column with high salt before application of methyl mannoside (sample b, Fig. 5B). However, washing the column with the detergent Empigen, as in the Fig. 4A chromatogram, resulted in a sharp elution of gp148 by methyl mannoside. The purity of the gp148 preparation is demonstrated in Fig. 5 (gp148, Fig. 5A and B). Fig. 4B shows UV spectra of the elution peaks from the chromatogram. The gp148-containing methyl mannoside fraction shows a typical protein spectrum

Table 2
List of lectins used

Abbreviation	Source	Main specificity
AAA	*Aleuria aurantia*	α(1-6)-linked Fuc
BSL-1	*Bandeiraea simplicifolia* seeds	α-GalNAc, α-Gal, Blgr.AandB
BSL-2	*Bandeiraea simplicifolia* seeds	α-/β-GlcNAc
Con A	*Canavalia ensiformis* (Jack been) seeds	α-Man
DBA	*Dolichos biflorus* (Horse gram) seeds	GalNAc(α-1,3)GalNAc, α-Gal, Blgr.A1
DSL	*Datura stramonium* (Thorn apple) seeds	GlcNAc(β-1,4)GlcNAc, terminal LacNAc-
ECL	*Erythrina cristagalli* (Coral tree) seeds	Gal(β-1,4)GlcNAc, GalNAc
GNA	*Galanthus nivalis* (Snow drop) bulb	terminal Man(α-1,3)Man-
JAC, jacalin	*Artocarpus integrifolia* (Jackfruit) seeds	O-linked Gal(β-1,3)GalNAc, α-Gal
LCA	*Lens culinaris* (lentil) seeds	α-Man
Lot	*Lotus tetragonolobus* (Asparagus pea)	α-L-Fuc, Blgr.H(0)
LPA	*Limulus polyhemus* (Horseshoe crab)	NANA, mucins, phosphorylcholin
MAA	*Maackia amurensis*	α(2-3)-linked NANA
PHA-E	*Phaseolus vulgaris* (red kidney bean)	Red cell specific saccharide
PHA-L	*Phaseolus vulgaris* (red kidney bean)	Lymphocyte specific saccharide
PNA	*Arachis hypogaea* (peanuts)	Gal(β-1,3)GalNAc-, Gal-
PSA	*Pisum sativum* (garden pea) seeds	terminal α-Man-, or α-Glc-
RCA-agl	*Ricinus communis* (castor beans) seeds	β-Gal-, β-GalNAc-
RCA-tox	*Ricinus communis* (castor beans) seeds	β-Gal-, βGalNAc-
SJA	*Sophora japonica* (Jap. pagoda tree) seeds	terminal GalNAc-, terminal Gal-
STL	*Solanum tuberosum* (potato) tubers	*oligo*-GlcNAc, MurNAc
UEA-I	*Ulex europaeus* (Furze gorse) seeds	α-Fuc, Blgr.A2, Blgr.H(0)
VVL	*Vicia villosa* (hairy vetch) seeds	GalNAc(α)-Ser/Thr, Blgr.A1,Tn
WGA	*Triticum vulgaris* (wheat germ)	GlcNAc(β-1,4)GlcNAc, NANA
sWGA	-succinylated WGA	terminal GalNAc(β-1,4)GlcNAc

Fuc = Fucose; Blgr. = blood group; Man = mannose; Lac = lactose; NANA = N-acetylneuramic acid (sialic acid); other abbreviations defined in text.

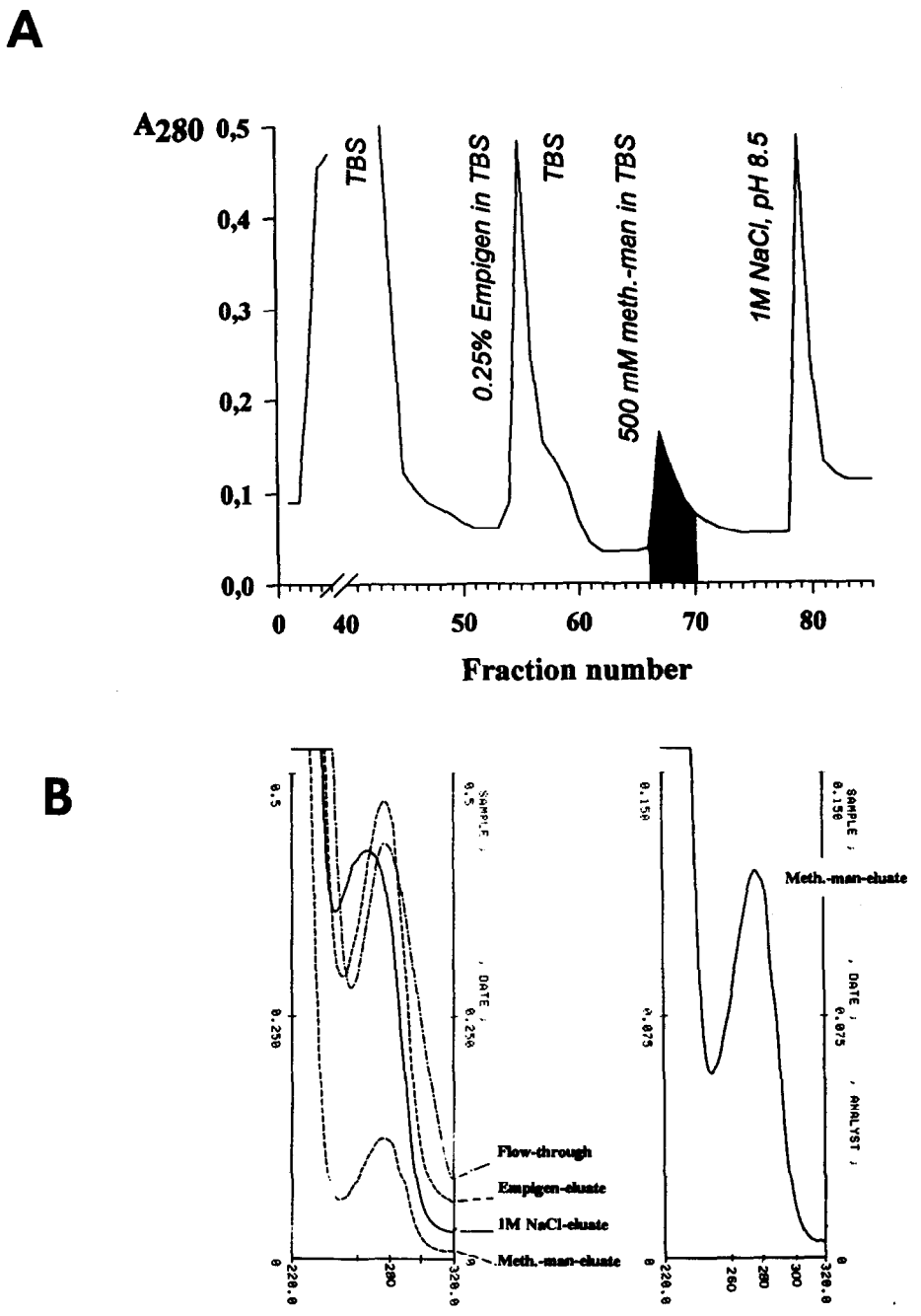

Fig. 4. Chromatography on ProSep-GNA, 30 × 8 mm. Sample: GlcNAc fraction obtained from the WGA column (see Experimental section). (A) Chromatogram showing the protein profile (A_{280}) and the buffer changes. The gp148 was found in the fraction eluted with 500 m*M* methyl mannoside (black). (B) The UV spectrum of the gp148 fraction (Meth.-man-eluate) is compared to those of the other fractions from the chromatogram shown in (A). To the right: The intensity axis is expanded to show the protein type spectrum of the gp148 fraction (Meth.-man-eluate).

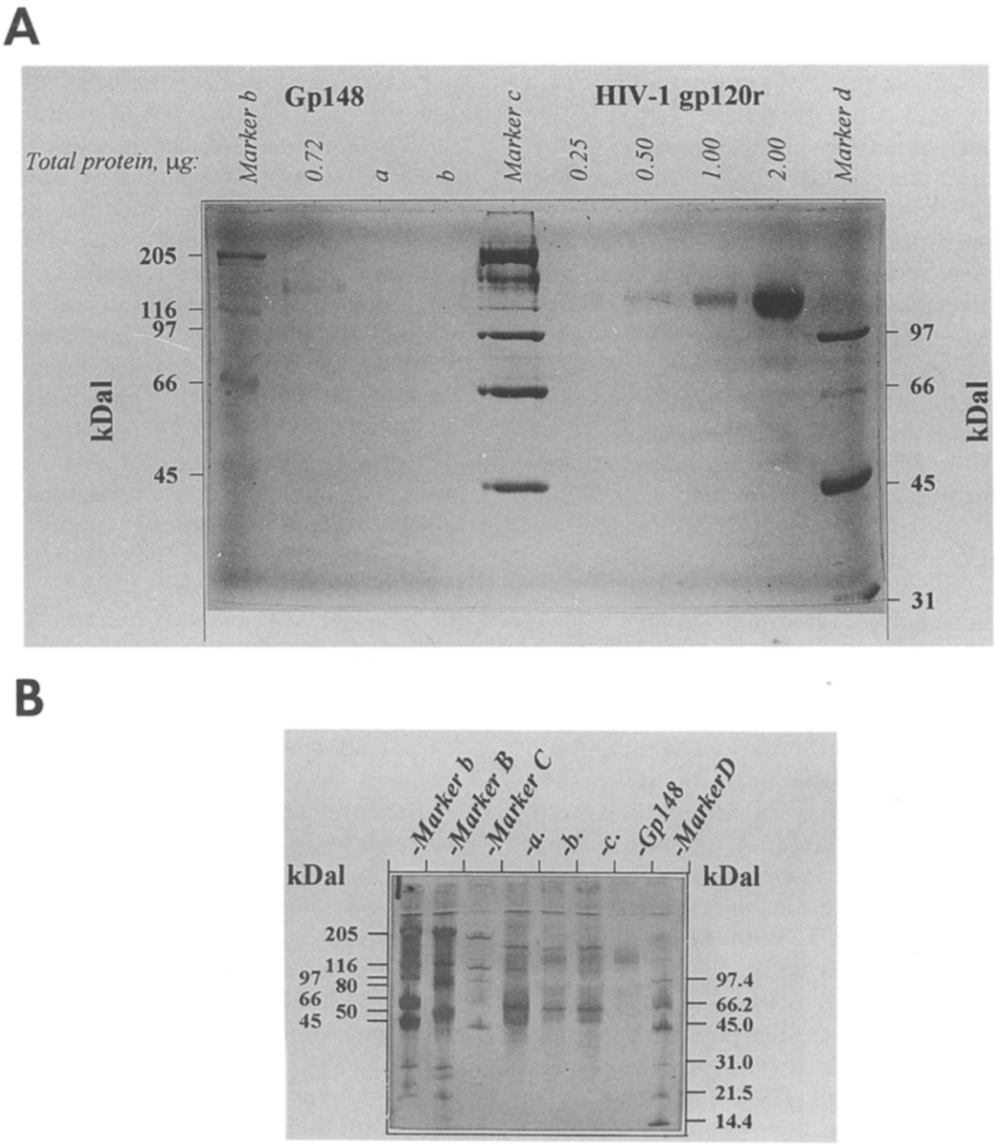

Fig. 5. (A) SDS-PAGE of the purified gp148 and a recombinant HIV-1_{IIIB}-gp120. The gel is stained with Commassie Brilliant Blue. The amount of applied protein is indicated above the gel. (B) SDS-PAGE (PhastSys, Pharmacia) with silver staining of the gel to show the peptide profile of purified gp148, obtained as the meth.-man fraction from ProSep-GNA chromatography according to the elution scheme in Fig. 4A. Sample a is the flow through from the same run. Sample b is the methyl mannoside fraction from a similar run in which the Empigen washing step was exchanged for by a wash with 1 *M* NaCl and sample c is the eluate obtained with 500 m*M* methyl mannoside in 1 *M* NaCl when the Empigen step was omitted.

(Meth.-man-eluate, Fig. 4B). A shoulder at about 290 nm may reflect the relatively high content of tryptophan relative to tyrosine (17:21) in the protein, as judged from the predicted amino acid sequence [19]. Material eluted with 1 *M* NaCl at elevated pH showed λ_{max} at 260 nm (Fig. 4B).

Protein content in the gp148 preparation was determined, with the same result, by UV spectroscopy and by the Bradford technique [20],

using thyroglobulin as standard. A comparison of staining intensity of the gp148 band in the SDS-PAGE gel with that of known amounts of a recombinant HIV-1 gp120 (rgp120) run in parallel (Fig. 5A), points to gp148 representing the full protein content in the applied sample. The silver stained gel in Fig. 5B, lane "gp148", shows that contaminants represent less than 1%. The purity is also revealed by the single band obtained in electroblot analyses with a large panel of lectins as probes (Fig. 6).

The recovery of the gp148 from the virus fraction was 45% and that obtained through the full purification procedure was about 10% (Table 1). Thus, from 25 l of infected cell culture about 1 mg of purified gp148 was obtained (Table 1).

3.4. Lectin blot analysis

Lectin blot analyses were done to give a view of the possible character of the oligosaccharides of gp148. These analyses (Fig. 6) point to the presence of high mannose type as well as complex type N-linked oligosaccharides in the gp148. This is in analogy with findings for SIV_{SM} [21] and HIV-1 [22–26]. As with SIV_{SM} [21] there seems to be ample presence of lactosamine containing saccharides. Hansen gives arguments and evidence for the presence of O-linked oligosaccharides in HIV [27]. With SIV gp148 the binding of jacalin and a weak signal from *Vicia villosa* lectin (JAC and VVL, Fig. 6, bottom right hand panel) indicate the possible presence of O-linked sugars. However, the peanut agglutinin blot was negative (PNA, Fig. 6, top right hand panel). Jacalin has been reported to block HIV-1 infection *in vitro* [28]. It was assumed not to bind to HIV-gp120, but to share similarities in its amino acid sequence with a stretch in the second conserved region of the glycoprotein and thereby exert its effect [28].

The lectin blot technique [15] may help in deciding on a suitable strategy for glycoprotein purification or analysis. The selectivity of GNA for gp148 among the glycoproteins of the solubilized virus fraction is evident from the lectin blot analysis (Fig 6, gna, bottom left hand panel). Other lectins that may be of use in the purification of the virus glycoprotein are those from *Erythrina cristagalli* (specificity for lactosamine) and *Aleuria aurantia* (specificity for α-fucose) (see Fig. 6, ECL and AAA, bottom left hand panel). However, both of these lectins also bind an $M_r \approx 100\,000$ glycopeptide as the main contaminant and are therefore not selective for gp148 to the same extent as the GNA.

GNA selectively binds terminal α-1,3-, and/or α-1,6-mannose residuals in oligosaccharides or in protein glycoconjugates [29,30]. Such structures, if present in mature extracellular proteins, are usually not exposed. Their presence in the external *env* protein seems to be a feature in common for different HIV isolates [31–34]. Consequently GNA, a similar lectin or mannan antibody, should constitute a general affinity method for the purification of these glycoproteins. This has been explored by Gilljam [12] for purification of the external *env* protein from HIV-1 and HIV-2 as well as from SIV.

4. Concluding remarks

In this paper we have advised a strategy for the purification of SIV_{MAC} from large volumes of culture fluid and for the subsequent fractionation of its sub-components. The good recovery of reverse transcriptase activity in the concentrated virus indicate a well preserved virion. The recovery of the external glycoprotein was not exceptional but may reflect the amount *de facto* attached to the envelope of the mature virus, as discussed above. HIV-1 gp120, when concentrated with a similar system, was recovered to a much higher extent (about 60%) [9]. However, this is a different virus and cell system and there may be differences in the kinetics of glycoprotein shedding from the cell surface and virus maturation [35].

Although the dextran–PEG system works well with several retroviruses [11] we have earlier found that other systems may be more advantageous when it comes to the recovery of the external *env* protein [7–9,11]. Therefore, it is possible that the extraction conditions can be better optimized for the SIV glycoprotein. How-

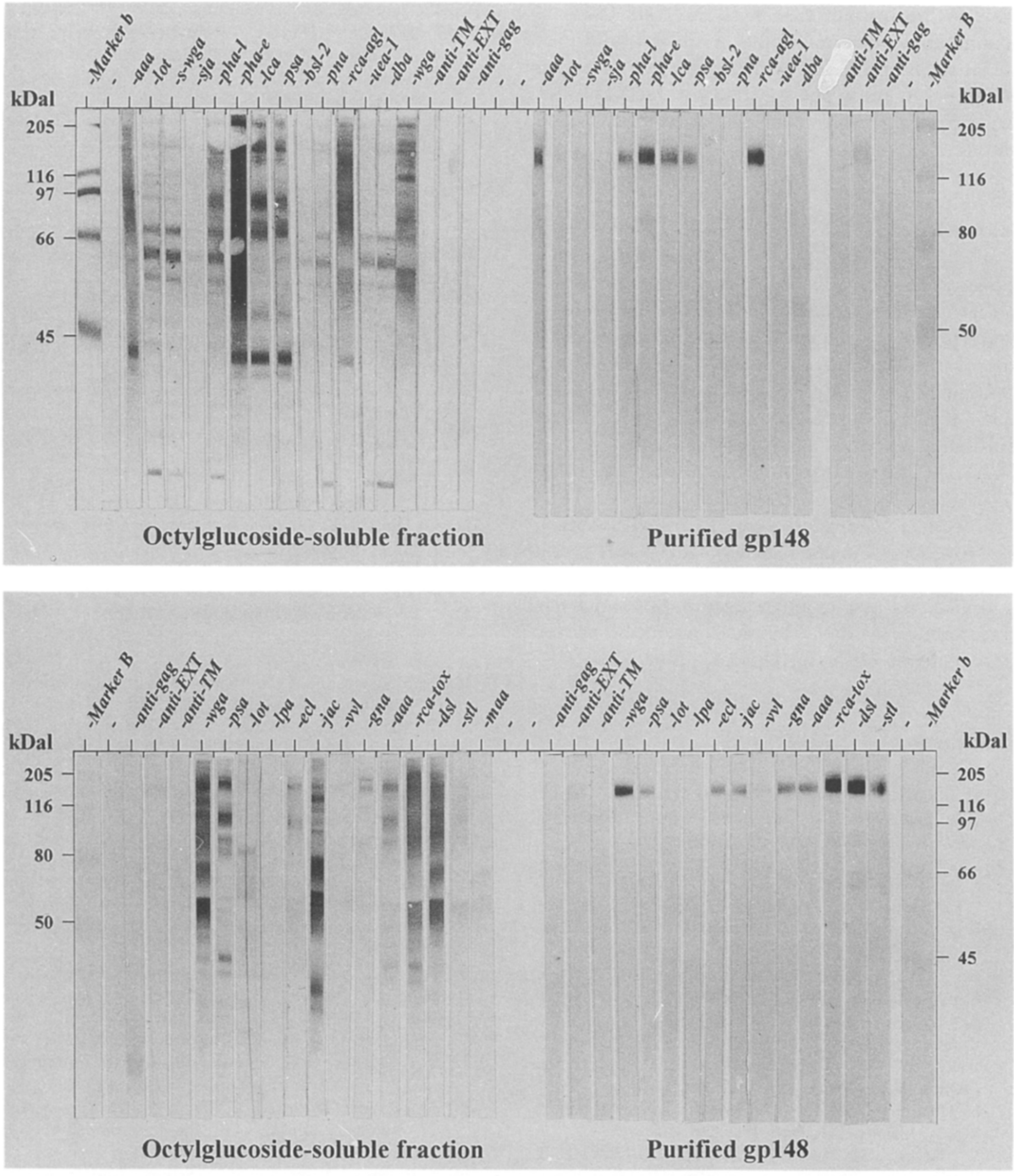

Fig. 6. Lectin and immunoblot analyses of octylglucoside soluble fraction (left-hand panels) from the extracted SIV preparation and of the purified gp148 (right-hand panels). Top and bottom panels show blots from two different SDS-PAGE gels. Markers are included at the outer borders. The probes are indicated above the blots. Lectin abbreviations are explained in Table 2.

ever, in the present case we appreciate the well documented biocompatibility and inert nature of the dextran and PEG, which ensures that traces of the polymers in the purified products will not cause major problems in the utilization of the prepared material.

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