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Octyl-β-D-Glucopyranoside Extracts Polyomavirus Receptor Moieties from the Surfaces of Mouse Kidney Cells†

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Polyomavirus receptor moieties were extracted from the surfaces of mouse kidney cells with the nonionic detergent octyl-β-D-glucopyranoside. Following extraction with this detergent, mouse kidney cells were refractory to polyomavirus infection. Binding studies demonstrated that this loss of susceptibility resulted from extraction of a peripheral membrane protein or proteins required for proper virus attachment to and infection of mouse kidney cells. Infection of extracted mouse kidney cells returned following a 2-h recovery period. However, the presence of cycloheximide or tunicamycin in the recovery media interfered with recovery from infection. Cells could be infected immediately after extraction by supplying them with the extracted moieties prior to or concomitant with infection. A complex of polyomavirus and the extracted receptor protein was formed by in vitro incubation and was stable in sucrose gradient analysis. Functional receptor moieties were prepared in the form of liposomes from the detergent extract. The virus-receptor complex was immunoprecipitated with anti-polyomavirus immunoglobulin G, and the portion of the complex contributed by the cell was identified. Immunoblot analysis of the mouse kidney cell detergent extract with a receptor-specific ^{125}I-labeled anti-idiotypic antibody or ^{125}I-labeled polyomavirus demonstrated several reactive proteins. Attachment of polyomavirus to mouse kidney cells, followed by extraction of the virus-receptor complex, identified polyomavirus-binding proteins similar to those observed in in vitro binding. Proteins with molecular weights of approximately 95,000, 50,000 and 25,000 to 30,000 were consistently observed in all receptor assays. The relationship between these proteins and their possible involvement as the cell receptor for polyomavirus are discussed.

Entry of viruses into cells can occur via several different mechanisms, most of which require interaction with a specific cellular receptor. While this interaction is not sufficient to guarantee successful virus entry, it is a necessary step. The specificity of virus-host interactions, therefore, relies heavily on the specificity of the virus attachment protein-cellular receptor interaction. Identification of cellular virus receptor moieties will be important for understanding mechanisms of virus infection and tissue tropism and for the development of antiviral agents.

Polyomavirus attaches to mouse kidney cells (MKC) via two physically and kinetically distinguishable binding mechanisms (3). Following attachment to the saturable, specific receptor, the virus is internalized, and infection ensues (8, 11, 16). Nonspecific binding results in virus internalization and eventual degradation in lysosomes (16). The receptor thus appears to play an important role in determining the course of polyomavirus infection which is to follow.

Although the polyomavirus attachment protein has been studied extensively (2, 12, 17), very little is known about the cellular receptor for polyomavirus. A previous report has suggested that a sialic acid protein modification of the form NeuAcα2,3Galβ1,3GalNAc is essential for a functional polyomavirus receptor on 3T6 cells and erythrocytes (9). Whether this is a ubiquitous modification found on polyomavirus receptors of all cell types is not known, and the backbone molecule to which sialic acid is attached has not been identified.

The nonionic detergent octyl-β-D-glucopyranoside (OG) has been used to solubilize peripheral cell surface proteins and lipids from a variety of isolated cell membranes as well as intact cells (15). This detergent allows the noncytolytic extraction of surface proteins and thus offers a distinct advantage over other nonionic detergents. In addition, OG is freely dialyzable to concentrations below the 25 mM critical micelle concentration (22). Prompted by successful OG extraction of biologically significant cell surface molecules by other investigators (15), we have taken advantage of this noncytolytic extraction procedure to extract and examine polyomavirus receptor moieties from the surfaces of MKC and to investigate the kinetics of receptor synthesis.

MATERIALS AND METHODS

Cell and virus propagation. Primary cultures of MKC were prepared as previously described (23) and maintained in Dulbecco-modified Eagle medium (DMEM) containing 10% fetal calf serum. Wild-type, small-plaque polyomavirus was used to infect cells at a multiplicity of infection of 10. Infected cultures were maintained in serum-free DMEM (18). Virions were purified from infected cell lysates as described previously (4–6, 18).

Anti-idiotypic antibodies. Polyclonal anti-idiotypic antibodies were isolated from rabbits (S. Marriott and R. Consigli, unpublished data) which had been immunized with a neutralizing monoclonal antibody (17). The anti-idiotypic antibodies were affinity purified and reacted specifically with the MKC receptor for polyomavirus. These antibodies were used to identify receptor moieties within the OG extract by immunoblot analysis.

Radioactive labeling. Purified polyomavirus virions or anti-idiotypic immunoglobulin G (IgG) was labeled in vitro with ^{125}I by the chloramine T method (10). The labeling reactions were quenched by the addition of unlabeled tyrosine to a final concentration of 0.1 mg/ml. Unbound ^{125}I was removed

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from all chloramine T-labeled substrates by centrifugation of the labeling mixture through a 2-ml Sephadex G-25 column at 12,000 × *g* for 10 min and overnight dialysis against 10 mM Tris hydrochloride (pH 7.4). MKC surfaces were labeled in vitro with ^{125}I by the lactoperoxidase method of Morrison (20).

OG extraction of MKC. Confluent MKC monolayers were washed with phosphate-buffered saline (PBS) and incubated in serum-free DMEM for 1 h at 37°C. The monolayers were washed again with PBS, and 1 ml of a 0.2% OG (Sigma Chemical Co., St. Louis, Mo.) solution was added. This solution was prepared in PBS and contained the protease inhibitors aprotinin, leupeptin, phenylmethylsulfonyl fluoride, *N*-*p*-tosyl-L-lysine chloromethyl ketone, and L-1-tosylamide 2-phenylmethyl chloromethyl ketone (Sigma Chemical Co.), each at a concentration of 10 μg/ml. The plates were incubated for 30 min at room temperature with periodic rotation before the OG extract was removed. For mock extractions, cells were incubated with PBS during this period. Extracted monolayers were washed twice with PBS, DMEM containing 10% fetal calf serum was applied, and the cells were incubated at 37°C. Multiple extractions of the cells were performed at 12-h intervals.

Extract solutions were centrifuged at 10,000 rpm for 30 min in a Sorvall SS-34 rotor to remove cell debris. For preparation of the soluble OG extract, the supernatant was concentrated to 1/10 the original volume and dialyzed overnight against a 10-fold volume of water. The soluble extract could be stored at −70°C for up to several months. Alternatively, for preparation of the OG extract in the liposome form, the supernatant was dialyzed against a 2,000-fold volume of 1 mM Tris hydrochloride (pH 7.4) and concentrated to 1/10 the original volume. The liposomal extract could be stored for up to 1 week at 4°C in the presence of the protease inhibitors described above.

Polyomavirus infection of extracted MKC. MKC were grown to confluency on glass cover slips. The monolayers were extracted as described above and allowed to recover for various periods of time in media containing 10% fetal calf serum. Following recovery, the cells were infected with 1,000 hemagglutination units of polyomavirus for 1 h at 4°C, washed, and incubated in serum-free DMEM at 37°C. At 40 h postinfection, the cover slips were fixed and stained for immunofluorescence (19). The degree of polyomavirus infection was determined by the percentage of fluorescing nuclei in the various cultures.

In studies performed with metabolic inhibitors, cycloheximide or tunicamycin (Sigma Chemical Co.) was included in the recovery media at a concentration of 5 μg/ml. Preliminary experiments demonstrated that this concentration of cycloheximide allowed complete inhibition of protein synthesis without causing cytopathic effects. Tunicamycin at 5 μg/ml allowed normal protein synthesis and also caused no cytopathic effects. Extracted cells were allowed to recover for various times from 0 to 4 h in media containing either cycloheximide or tunicamycin. At the designated intervals, the recovery media were removed, and the cells were washed. Infection and immunofluorescence staining were performed as described above.

Reconstitution of virus susceptibility in extracted cells. OG-extracted MKC surface moieties were reinserted into extracted cells by either of two methods. First, 10 μg of OG extract protein in the liposome form was incubated with 10^6 extracted cells in 1 ml of PBS for 1 h at 37°C. Following this incubation, the cells were infected. Second, purified polyomavirus virions were mixed with 10 μg of liposomal OG extract protein in vitro for 1 h at room temperature. Following this reaction, the virion-OG extract complexes were used to infect 10^6 extracted MKC. The degree of infection was determined by immunofluorescence staining 40 h postinfection as described previously (19).

Binding of polyomavirus to extracted MKC. Confluent monolayers of MKC were either extracted or mock extracted as described above. Following extraction, the cells were placed on ice and washed with PBS. The cells were reacted with 0.1 ml of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) binding buffer (0.5 M HEPES [Calbiochem-Behring, La Jolla, Calif.], 8 mM glucose, 1.6 mM bovine serum albumin, 0.15 M NaCl, 4.9 mM KCl, 0.6 mM $MgSO_4$ [pH 7.3]) containing 10^5 cpm (20 ng of protein) of ^{125}I-labeled polyomavirus virions. The binding reaction proceeded for various times from 0 to 4 h after extraction at 4°C, after which the monolayers were washed with PBS and the cells were dissolved in 0.5 N NaOH. The cell-associated counts were determined with an LKB 1275 Minigamma Counter.

Recovery of virus-binding ability. An additional binding experiment was performed to investigate the ability of extracted cells to recover virus-binding ability. MKC monolayers were extracted and allowed to recover for various periods of time. At the designated intervals, the cells were placed on ice, the recovery media were removed, and the cells were washed with cold PBS. Binding buffer containing 10^5 cpm of ^{125}I-labeled virus was added to these cells for 2 h following the recovery interval. The cell-associated counts were determined as described above.

Sucrose gradient analysis of polyomavirus-OG extract complexes. MKC OG extract in the liposome form (10 μg) was mixed with 10^6 cpm of ^{125}I-labeled polyomavirus virions in 100 μl of binding buffer for 1 h at room temperature. Following this incubation, ^{125}I-labeled virus alone or the virus-OG extract complex was placed on a preformed 10 to 30% linear sucrose gradient containing 10 mM Tris hydrochloride (pH 7.4), 0.15 M NaCl, and 0.01% Triton X-100. The gradient was centrifuged for 40 min at 33,000 rpm in a Beckman SW50.1 rotor at 4°C. Fractions of the gradient were collected by downward displacement with mineral oil. ^{125}I-labeled virus was located in the gradient by counting in an LKB 1275 Minigamma Counter.

Electron microscopy. Polyomavirus-OG extract complexes were isolated from a 10 to 30% sucrose gradient peak. The samples were placed on carbon-coated grids and stained with 2% uranyl acetate. The grids were examined and photographed in a Philips 201 electron microscope.

Immunoprecipitation. MKC monolayers were extracted with OG as described above. Following extraction, the cells were labeled in vivo for 4 h with 10 μCi of a ^{14}C-amino acid mixture (New England Nuclear Corp., Boston, Mass.) per ml in media containing 10% fetal calf serum and 10% amino acids. The cells were extracted a total of four times, and the ^{14}C-amino acid-containing media were replaced after each extraction. The extract was concentrated as descibed above to form the soluble OG extract. Polyomavirus-OG extract complexes were formed in vitro as described above with 10 μg of purified polyomavirus virions and 155,000 cpm of ^{14}C-labeled soluble OG extract. The complexes or ^{14}C-labeled OG extract alone was immunoprecipitated with normal rabbit IgG to remove nonspecific IgG binding, followed by rabbit anti-polyomavirus IgG as described previously (17, 24). The precipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fluorography to detect ^{14}C-labeled proteins was

performed as described by Chamberlain (7), and autoradiography with Kodak XAR-5 X-ray film with a Cronex Lightning-Plus intensifying screen was used to detect the labeled proteins.

Immunoblot analysis. OG extract proteins in the soluble form were separated by 15% SDS-PAGE and transferred to a nitrocellulose membrane (Schleicher & Schuell, Inc., Keene, N.H.) as described by Bittner et al. (1). The residual binding capacity of the nitrocelluose was blocked by incubation overnight with TDN buffer (10 mM Tris hydrochloride [pH 7.5], 0.05 M NaCl, 2 mM disodium EDTA · $2H_2O$) containing 4% bovine serum albumin and 10 μg of normal rabbit IgG per ml. The proteins were probed directly with ^{125}I-labeled polyomavirus virions, anti-idiotypic IgG, or normal rabbit IgG in TDN buffer containing 4% bovine serum albumin and 10 μg of normal rabbit IgG per ml for 12 h. After being washed, the blots were exposed to Kodak XAR-5 X-ray film with a Cronex Lightning-Plus intensifying screen.

OG extraction of polyomavirus-receptor complexes formed in vivo. MKC (10^6) which had been surface ^{125}I-labeled by the lactoperoxidase method (20) were incubated with 10 μg of purified polyomavirus virion protein for 1 h at 4°C. Unlabeled MKC (10^6) were mock surface labeled and incubated with 10 μg of ^{125}I-labeled polyomavirus virion protein (3×10^6 cpm) for 1 h at 4°C. The cells were washed with PBS and extracted with OG as described above. The soluble extract was centrifuged to remove cell debris and placed immediately on a 10 to 30% sucrose gradient. The gradient was centrifuged for 40 min at 33,000 rpm in a Beckman SW50.1 rotor. Fractions were collected, and peaks were identified by counting in an LKB 1275 Minigamma Counter. Peak fractions were dialyzed to remove sucrose and concentrated to 50 μl. The concentrated samples were analyzed by 15% SDS-PAGE (11), followed by silver staining (21) and autoradiography.

RESULTS

OG was used to extract receptor moieties required for polyomavirus infection from the surfaces of MKC. In contrast to previous studies performed by extracting cells in suspension (15), we have demonstrated that extraction of cells in a monolayer is also possible. The standard extraction conditions for cells in a monolayer, 7 mM (0.2%) OG at 23°C for 30 min, yielded approximately 5 μg of protein per 10^6 cells (data not shown). This yield is in the lower range of that reported for cells in suspension (15) and probably reflects the fact that for cells in a monolayer, only half of the cell surface is accessible to detergent.

Infection of OG-extracted cells. To investigate the involvement of an OG-extractable moiety in polyomavirus infection, we measured infection with and without OG extraction (Fig. 1). Normal, unextracted MKC routinely showed approximately 80% infection after 40 h under the conditions used. When infected immediately following extraction, however, cells supported less than 10% infection, indicating that extraction reduces infection by approximately 70%. Incubation of extracted cells in media containing 10% fetal calf serum allowed the cells to replace the extracted moieties, resulting in an increased amount of infection dependent upon the length of the recovery period. Following a 2-h recovery, the number of infected cells observed in extracted cultures was not significantly different from the 80% infection observed in unextracted cultures (Fig. 1). It thus appears that MKC are able to replace an OG-extractable cell surface moiety required for polyomavirus infection within 2 h.

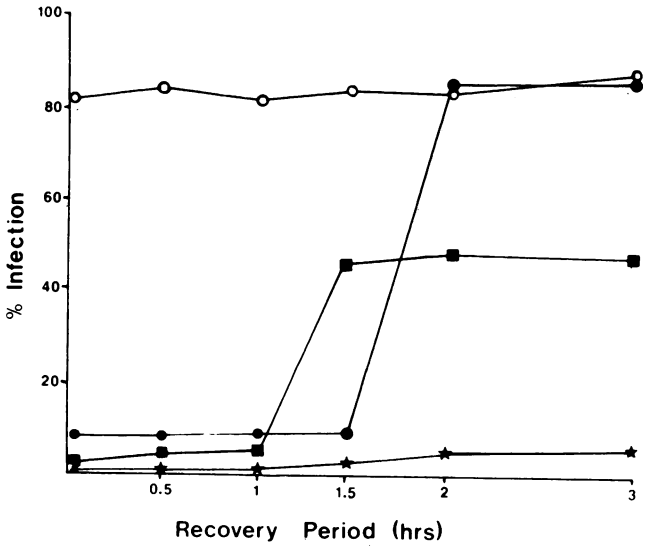

FIG. 1. Recovery of infectibility in OG-extracted MKC. MKC were OG extracted and allowed to recover for various periods of time in normal recovery media (●), media containing 5 μg of cycloheximide per ml (■), or media containing 5 μg of tunicamycin per ml (★). Infection of cells which were treated with a mixture of polyomavirus and liposomal OG extract at various times after extraction was identical to infection of unextracted cells and is also indicated (○). After the designated recovery periods, the cells were infected. Infection proceeded for 40 h, at which time the cells were fixed and stained for immunofluorescence. Percent infection was determined by comparing the total number of nuclei with the number of fluorescing nuclei.

Lack of infection in cells up to 2 h postextraction was not a result of metabolic injury to the cells. Infection could be obtained in these cells, even immediately following extraction, by supplying extracted cells with a combination of polyomavirus and OG extract in the liposome form (Fig. 1). OG extract could also be incubated with extracted cells and washed off before virus was added for infection. Cells treated in this manner were also infectible immediately following extraction (data not shown). These results are consistent with the hypothesis that OG-extracted cells lack a cell surface moiety required for polyomavirus infection and that extracted cells supplemented with a source of the receptor moiety can support virus infection.

Inhibition of recovery of virus susceptibility. Two metabolic inhibitors were used to determine whether the extractable portion of the polyomavirus receptor was replaced from intracellular protein pools or whether resynthesis was required. When cycloheximide was included in the media during the recovery period, infection remained below 50% up to 3 h after adsorption (Fig. 1). When tunicamycin was included in the recovery media, infection remained below 10% up to 3 h after adsorption (Fig. 1). These results suggest that protein synthesis and glycosylation are required to fully replace the extractable portion of the MKC receptor for polyomavirus. Thus, recycling or recruitment of completely functional receptors from internal protein pools is not occurring.

Virus binding to extracted cells. A virus-binding experiment was performed to confirm the OG extraction of a cell surface moiety required for polyomavirus attachment to MKC. This experiment (Fig. 2) demonstrated that following extraction, virus binding was reduced by 50% from that in

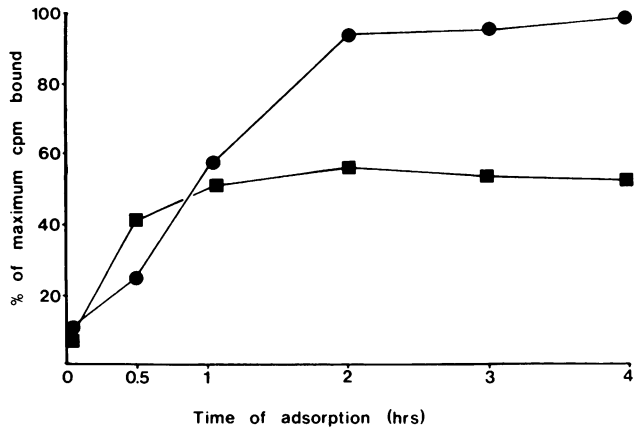

FIG. 2. Binding of polyomavirus virions to OG-extracted MKC. MKC were either OG extracted (■) or mock extracted (●) and incubated with ^{125}I-labeled polyomavirus virions for various periods of time. At the designated intervals, the cells were solubilized with 1 N NaOH, and cell-associated counts were determined.

normal cells, thus indicating a 50% reduction in the number of polyomavirus receptors on the surfaces of extracted cells. Maximal virus binding was observed after a 2-h adsorption period; thus, this time was chosen to investigate the recovery of virus-binding ability in extracted cells. The ability of extracted cells to bind virus returned gradually until binding equaled that in unextracted cells at 4 h after extraction (data not shown). These results suggest that the extracted receptor moiety is involved in virus binding and that the loss of infectibility in extracted cells may be related to the reduction in virus binding.

Formation of virus-OG extract complexes. Figure 1 illustrates that a mixture of polyomavirus and OG extract could infect cells immediately after extraction. Therefore, the polyomavirus-OG extract mixture was examined more closely. Binding of polyomavirus to a component of the liposomal OG extract was observed following sucrose gradient analysis of the virus-extract complex (Fig. 3). In this gradient, a ^{125}I-labeled virus marker migrated at fraction 10 (Fig. 3B) and identified the sedimentation position of 240S uncomplexed virus. However, following mixture with liposomal OG extract, a population of labeled virus migrated more slowly in the gradient (Fig. 3A). Electron microscopy of the polyomavirus-OG extract complex isolated from fractions 22 to 26 of the sucrose gradient shown in Fig. 3A demonstrated small, homogeneous liposomes with an average diameter of 300 nm and coated with polyomavirus virions (Fig. 3C). Apparently, polyomavirus was capable of binding preferentially to the liposome form of the OG extract, which had incorporated the virus-binding portion of the cellular receptor for polyomavirus in the correct orientation, causing the labeled virus complexed with liposomes to be more buoyant in the gradient.

Immunoprecipitation of virus-OG extract complexes. After demonstrating the presence of polyomavirus receptor moieties in MKC-derived liposomes, we wanted to identify individual virus-reactive proteins within the soluble form of the OG extract. Soluble OG extract prepared from cells which had been labeled in vivo with ^{14}C-amino acids was mixed with polyomavirus virions in vitro to form virus-OG extract complexes. These complexes or soluble OG extract alone was immunoprecipitated with anti-polyomavirus IgG, and the precipitates were analyzed by SDS-PAGE (Fig. 4). Of the total OG extract proteins (Fig. 4C), proteins with molecular weights of 50,000 and 25,000 to 30,000 were precipitated from the virus-OG extract complexes (Fig. 4B). A protein with a molcular weight of 18,000 to 20,000 was occasionally observed in some preparations (Fig. 4B) but not routinely observed in all preparations. No proteins were precipitated with anti-polyomavirus IgG from the soluble OG extract alone (Fig. 4A).

Immunoblot reactivity of OG extract proteins. A receptor-specific polyclonal anti-idiotypic antibody was used to identify possible receptor proteins within the OG extract (Fig. 5). This anti-idiotypic antibody has been shown to block polyomavirus attachment to a protein component of the MKC receptor for polyomavirus (Marriott and Consigli,

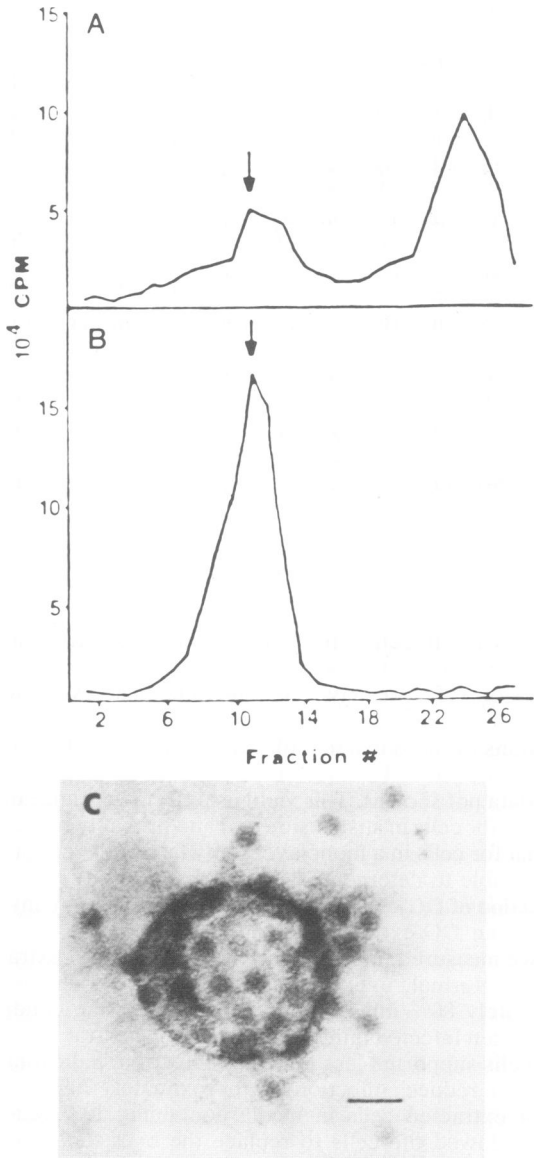

FIG. 3. Sucrose gradient analysis of polyomavirus-OG extract complexes formed in vitro. ^{125}I-labeled polyomavirus virions alone (B) or following reaction with OG extract in the liposome form (A) were placed on 10 to 30% linear sucrose gradients. The arrows indicate the migration of the 240S virus marker. The sedimentation is from right to left. Fractions 22 to 26 of panel A were pooled and examined by electron microscopy (C). Bar, 100 nm.

unpublished data) by mimicking the polyomavirus attachment protein. In an immunoblot assay, both polyomavirus virions (Fig. 5B) and the anti-idiotypic antibody (Fig. 5C) recognized specific OG extract proteins as compared with the total OG extract proteins (Fig. 5D). These proteins had apparent molecular weights of 95,000, 50,000, and 25,000 to 30,000 and appeared similar to membrane proteins recognized by anti-idiotypic antibodies. Also, similar proteins were recognized by an anti-120-kilodalton protein antibody directed aginst the cross-linked virus attachment protein-receptor complex (12). A protein with a molecular weight of 18,000 to 20,000 was occasionally observed in some preparations (Fig. 4C) but not routinely seen in all preparations. No proteins were recognized by normal rabbit IgG (Fig. 5A).

Previous investigators have suggested that specific binding of polyomavirus to 3T6 cells requires a NeuAcα2,3Galβ1, 3GalNAc sialic acid modification. We investigated the possibility that polyomavirus attachment to MKC receptors may also require a sialic acid modification. Soluble OG extracts were treated with neuraminidase to remove sialic acid residues and compared with untreated extracts on an immunoblot. No differences in reactivity were observed between normal and neuraminidase-treated extracts with either the virus or the anti-idiotype antibody as a probe (data not shown). This result demonstrates that sialic acid modification does not appear to play a major role in the attachment of polyomavirus to OG extract receptor proteins.

In vivo extraction of virus-receptor complexes. Polyomavirus-receptor complexes formed in vivo were extracted from intact MKC monolayers and subjected to sucrose gradient analysis. ^{125}I-labeled virus was used to form virus-receptor complexes. When these complexes were subjected to sucrose gradient analysis, a single peak migrating at fraction 15 was observed (Fig. 6B). This peak was used as a marker to identify virus-receptor complexes when ^{125}I-cell surface-labeled MKC were used to form virus-receptor complexes and subjected to sucrose gradient analysis (Fig. 6A). A small peak migrating with the marker peak was observed in Fig. 6A and identified as virus-receptor complexes containing cell surface label. This peak was analyzed by SDS-PAGE, and proteins with appaernt molecular weights of 95,000, 50,000, and 25,000 to 30,000 were identified (Fig. 7B) in the total ^{125}I-labeled OG extract (Fig. 7C). No MKC proteins were detected in the same region of the gradient when OG extract from uninfected cell surface-labeled cells was analyzed (Fig. 7A).

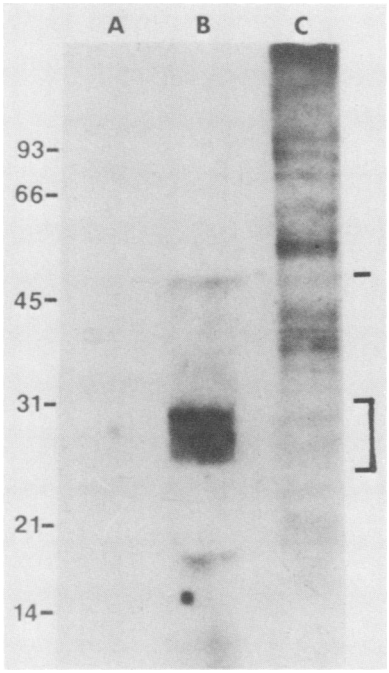

FIG. 4. SDS-PAGE analysis of immunoprecipitated polyomavirus-OG extract complexes. Soluble OG extract prepared from MKC labeled with ^{14}C-amino acids was mixed with polyomavirus virions. The soluble OG extract alone (lane A) or polyomavirus-OG extract complexes (lane B) were immunoprecipitated with anti-polyomavirus IgG. The precipitates were analyzed by 15% SDS-PAGE, followed by fluorography and autoradiography. The total ^{14}C-labeled OG extract is shown in lane C. Numbers represent molecular weights in thousands. Line and bracket at right of figure indicate positions of reactive proteins.

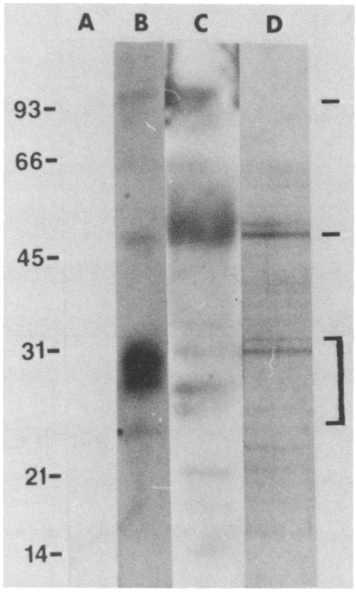

FIG. 5. Immunoblot identification of polyomavirus receptor moieties in OG extracts. OG extract proteins were separated by 15% SDS-PAGE and stained with Coomassie blue (lane D) or transferred to a nitrocellulose membrane. The membranes were probed with ^{125}I-labeled normal rabbit IgG (lane A), ^{125}I-labeled polyomavirus virions (lane B), or ^{125}I-labeled anti-idiotype IgG (lane C). Reactive proteins were identified by autoradiography. Numbers represent molecular weights in thousands. Lines and bracket at right of figure indicate positions of reactive proteins.

DISCUSSION

It has been particularly difficult to identify components of the polyomavirus receptor because of their relatively low abundance on the cell surface. Bolen and Consigli have previously reported that MKC possess approximately 10,000 polyomavirus-specific receptors per cell (3). If we assume that the receptor has a molecular weight of 50,000, we calculate that 10^6 MKC would contain 8×10^{-4} pg of receptor protein. Since we have suggested that our extraction procedure removes approximately 50% of the receptors, at best we could obtain 4×10^{-4} pg of receptor protein per 10^6 cells. Our total OG extract contained approximately 5 μg of protein per 10^6 cells, indicating that our extraction procedure is not specific only for polyomavirus receptors but

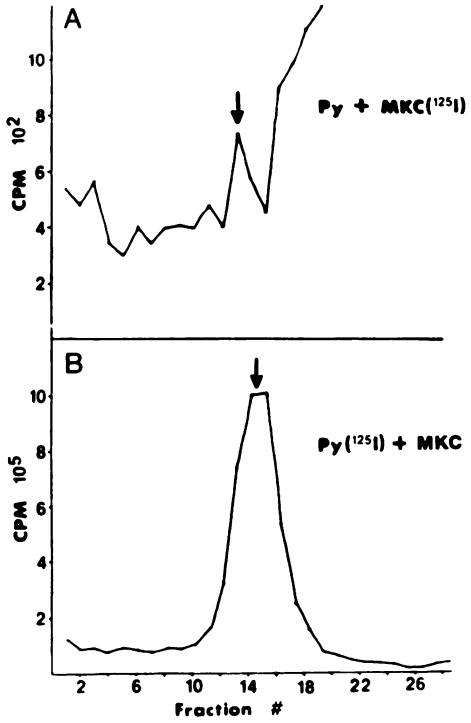

FIG. 6. Sucrose gradient analysis of polyomavirus (Py)-OG extract complexes formed in vivo. ^{125}I-labeled polyomavirus virions were allowed to adsorb to unlabeled MKC (B), or unlabeled polyomavirus virions were allowed to adsorb to ^{125}I-cell surface-labeled MKC (A). Following adsorption, the virus-receptor complexes were extracted and placed on 10 to 30% linear sucrose gradients. The arrows indicate the positions of virus-receptor complexes in the gradient. The bottom of the gradient is to the left.

rather that receptor moieties are extracted along with many other proteins which fall into the class of peripheral membrane proteins. Because of these considerations, our attempts to identify the MKC receptor for polyomavirus have relied on the use of receptor-specific antibodies to identify the receptor by immunoblot analysis or some type of radioactive label incorporated into MKC proteins to identify the receptor by autoradiography.

In addition to peripheral membrane proteins, OG extraction of cells removes certain lipids from the cell surface. It appears likely that there is some lipid associated with the receptor proteins identified in this study. We have shown that virions attach to OG extract in the liposome form (Fig. 3C). We have also observed that purified polyomavirus virions migrate faster in the sucrose gradient (Fig. 3B) than do virions complexed with OG extract in the soluble form (Fig. 6B), indicative of lipid or lipoprotein association with the virions. It thus appears that proteins and lipids may both be required and interact in concert to obtain a fully functional polyomavirus receptor. Further work will be necessary to identify specific lipid requirements for receptor activity.

Both cycloheximide and tunicamycin were capable of interfering with the return of infectivity in extracted MKC. Cycloheximide inhibited infection by 50% following a 2-h recovery period. We speculate that the extracted receptor moieties are present in low amounts sequestered within intracellular protein pools. The infected cells observed after a 2-h recovery period in the presence of cycloheximide probably resulted from insertion of the receptor moieties from intracellular protein pools into the host membrane, thus constituting functional receptors. In the case of tunicamycin, interference with infection appeared to be complete, and less than 10% of the cells could be infected even after a 3-h recovery period. Two possible explanations for this observation exist. First, the intracellular receptor moiety pools may lack or have incomplete glycosylation. Therefore, even though 50% of the cells have the correct receptor proteins on their surfaces and thus can bind virus, they lack the specific glycosylation which makes them capable of internalizing the particles for infection. Second, 50% of the cells may have fully functional receptors on their surfaces, but a glycosylation event may be required at the time of binding of virus to its specific receptor. This glycosylation may be required to stabilize the virus-receptor union and possibly to signal other early events of virus infection such as internalization or transport to the nucleus or both. Therefore, cells which lack correct glycosylation do not become infected.

The binding assays which we performed detected total virion attachment to both specific receptors and nonspecific binding moieties, whereas infection results solely from virion attachment to specific receptors. As shown in Fig. 1, 70% of the infection observed in normal cells was absent in cells immediately following extraction. However, virion binding immediately after extraction was reduced only 50%

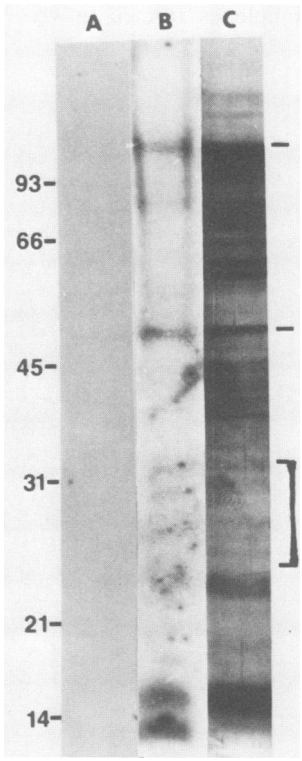

FIG. 7. SDS-PAGE analysis of polyomavirus-OG extract complexes formed in vivo. 15% SDS-PAGE was used to analyze gradient peaks from Fig. 6. Shown are ^{125}I-cell surface-labeled virus-receptor complexes derived from fractions 14 to 18 of the sucrose gradient in Fig. 6, panel A (lane B), ^{125}I-labeled OG extract alone from the same region of a separate gradient (lane A), and the total ^{125}I-labeled OG extract (lane C). The labeled proteins were detected by autoradiography. Numbers represent molecular weights in thousands. Lines and bracket at right of figure indicate positions of reactive proteins.

from that observed in unextracted cells (Fig. 2). From this information we can speculate that OG extraction of MKC removes polyomavirus-specific receptors more efficiently than it removes nonspecific binding moieties. In addition, our laboratory has previously demonstrated that the specific attachment of polyomavirus to MKC appears to be neuraminidase resistant, while nonspecific attachment appears to be sensitive to neuraminidase treatment (3). We have observed that neuraminidase treatment of cells prior to extraction or treatment of soluble OG extract itself does not significantly alter the immunoblot reactivity of extracted proteins (data not shown), suggesting that these reactive proteins are associated with specific binding of polyomavirus to MKC.

A 4-h recovery period is required to completely replace extracted receptor moieties from the surfaces of MKC, as determined by binding assays (data not shown). However, the ability of the cells to become infected is recovered within 2 h after extraction. It appears that the normal number of receptors on the surfaces of MKC is in excess of the number required for infection. The binding experiments demonstrated that 80% of the receptors capable of binding virus in normal cells are present in the cell membrane and are capable of binding virus 2 h after extraction. If specific and nonspecific receptors are regenerated at an equivalent rate, then approximately 8,000 specific receptors are required before infection can occur. Of course, this number probably reflects the low probability of a virus coming in contact with any given receptor, since it is theoretically possible for only a single internalized virus particle to initiate infection.

We feel that polyomavirus receptors are extracted from all cells in the monolayer during OG treatment but that in some cells the number of receptors remaining on the surface is still above the threshold level required to obtain infection of those cells. It is this fraction of the cells (approximately 10%) whose specific receptor number is above the threshold level following extraction and which can become infected immediately after extraction.

We have used several different experimental approaches to identify OG-extracted polyomavirus receptor moieties on the surfaces of MKC. We were able to bind virus directly to components of the OG extract (Fig. 3) and to identify the virus-reactive soluble OG extract components by immunoprecipitation (Fig. 4). Also, direct virus binding was used in an immunoblot to identify virus-reactive OG extract components (Fig. 5B). Proteins which reacted with receptor-specific anti-idiotypic antibodies in an immunoblot (Fig. 5C) had molecular weights similar to those of proteins recognized by virus in this assay. In addition to these approaches, which involved in vitro attachment of polyomavirus or anti-idiotypic antibodies to OG-extracted components, we also investigated the proteins to which polyomavirus binds in vivo (Fig. 6 and 7). These assays have consistently identified proteins with molecular weights of 95,000, 50,000, and 25,000 to 30,000 which are extracted from MKC by OG and which have polyomavirus receptor activity in various assays. The presence of protease inhibitors in the extract preparation reduces the possibility that these proteins represent proteolytic degradation products of a larger receptor protein and increases the probability that these proteins represent subunits of a larger receptor protein or protein complex. All of our assays utilized the denaturing conditions of SDS-PAGE to identify individual receptor components, but it is evident that future studies must involve nondenaturing gel systems to identify the receptor complex in its native conformation. Thus far, we have been unsuccessful in attempts to analyze the receptor moieties by conventional nondenaturing gel systems. It appears that the receptor moieties are too large to enter the gels, again suggesting that the moieties which we have identified are subunits of a large receptor complex. The adenovirus-receptor interaction also appears to involve more than one plasma membrane protein (13).

The ability to extract the MKC receptor for polyomavirus, leaving the cells metabolically active, will allow us to perform detailed studies of receptor synthesis and composition as well as to investigate the physiologic effects of the virus-receptor union. In addition, our ability to incorporate receptors with functional binding activities into liposomes derived from the host cell may provide an important biological system for studying virus-receptor interactions.

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