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VP7 : an attachment protein of bluetongue virus for cellular receptors in *Culicoides variipennis*

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The importance of VP7 of bluetongue virus (BTV) in the binding of BTV to membrane proteins of the BTV vector *Culicoides variipennis* was investigated. Core BTV particles, prepared from whole viruses, lacked outer proteins VP2 and VP5 and had VP7 exposed. More core particles and whole viruses bound to membrane preparations of adults of *C. variipennis* and KC cells, which were cultured from this vector insect, than to membrane preparations of *Manduca sexta* larvae. More core particles than whole viruses bound to membrane preparations of adults of *C. variipennis* and KC cells. Polyclonal anti-idiotypic antibodies (anti-Id), which were made against an antigen-combining region of an anti-BTV-10 VP7 antibody and functionally mimicked VP7, bound more to the membrane preparations of adults of *C. variipennis* and KC cells, and less to cytosol preparations. In Western overlay analysis, the *Culicoides* plasma membrane preparation reduced binding of an anti-VP7 monoclonal antibody to VP7. Whole and core BTV particles and the anti-Id bound to a membrane protein with a molecular mass of 23 kDa that was present predominantly in membrane preparations of adults of *C. variipennis* and KC cells. This protein was present in much lower concentrations in membrane preparations of C6/36 and DM-2 insect cells.

Introduction

Bluetongue disease is an infectious, non-contagious, arthropod-borne viral disease of domestic and wild ruminants that causes substantial economic losses to the United States and world livestock industry. This disease is caused by bluetongue viruses (BTV), which are orbiviruses that are transmitted by various biting midges of the genus *Culicoides* (Diptera : Ceratopogonidae). The major insect vector species of BTV in North America is *C. variipennis* of which the subspecies *C. variipennis sonorensis* is considered to be the most important in BTV transmission (Sellers, 1981; Jones *et al.*, 1981; Tabachnick, 1996).

Vector–virus interactions play a crucial role in vector-borne disease epidemiology. The rate of infection of the vector by the pathogen is an important factor (Anderson & May, 1991). Bluetongue disease is present only where there are suitable vectors (Tabachnick, 1996; Tabachnick & Holbrook, 1992; Walton *et al.*, 1992). The initial requisite for productive virus infection of the vector is recognition and binding of the virus to midgut epithelial cells. These processes may determine the host range and cell/tissue tropism of the virus, and thus could be important components for vector competence. *C. variipennis* females are infected with BTV by imbibing viraemic blood from an infected vertebrate host. If the midge is susceptible to infection with the virus, the virus attaches to and enters the luminal surface of the midgut cells and replicates in them. Progeny viruses are released through the basement lamina into the haemocoel where they circulate with the haemolymph and infect secondary target organs, including the salivary glands (reviewed by Mellor, 1990). The infected, competent vector transmits the virus by feeding on the blood of susceptible hosts.

The mechanisms by which BTV attaches to midgut cells of the vector are poorly understood, even though the structures of BTV particles and BTV proteins are well-documented (Verwoerd *et al.*, 1972; Hewat *et al.*, 1992; Grimes *et al.*, 1995). Intact BTV is an icosahedral particle ca. 85 nm in diameter, composed of ca. 80% protein and 20% double-stranded RNA

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(dsRNA). The dsRNA has ten segments which encode seven structural and four non-structural proteins. The structural proteins are numbered VP1 to VP7 based on their molecular sizes and electrophoretic migration in polyacrylamide gels (PAGE). The non-structural proteins are designated NS1, NS2, NS3 and NS3a. The outer capsid, composed of VP2 and VP5, surrounds a core particle composed of 260 trimers of VP7 and 120 copies of VP3. Enclosed in the inner core are the remaining structural proteins and the dsRNA genome.

The genes encoding VP2 and VP5 show the greatest genetic variation among BTV serotypes and VP2 contains the serotype-specific determinants (Mecham *et al*., 1986; Roy, 1992). The proteins in the outer capsid layer are often considered to be crucial for virulence and recognition by host cells (Cowley & Gorman, 1986, 1989; Mertens *et al*., 1989; Huismans *et al*., 1983, 1987*a*, *b*, *c*; Roy *et al*., 1990). Core particles lacking VP2 and VP5, however, are just as infectious for the insect vector, *C. variipennis*, as intact virus particles, and even more infectious for cultured cells from this species (Mellor, 1990; Mertens *et al*., 1987, 1996). The finding that the VP7 protein is accessible to the surface of an intact virus (Lewis & Grubman, 1990) suggests that VP7 may be important in interactions of the virus with insect cells. We report here evidence for the involvement of VP7 in the binding of BTV to membrane preparations of vector insects and cultured cells.

Methods

■ **Insects.** The laboratory AK colony of the subspecies *C. variipennis sonorensis* (Mecham & Nunamaker, 1994) was established in 1973 from insects trapped in Owyhee County, Idaho, USA (Jones & Foster, 1978). Fifth instar larvae of *Manduca sexta* and adults of *Drosophila melanogaster* were raised from eggs purchased from Carolina Biological Supply Co., Burlington, NC, USA, on artificial diets obtained from the same company.

■ **Viruses.** The viruses used for this study were BTV serotype 10 (BTV-10; BT-8 strain) and BTV serotype 11 (BTV-11; Station strain). Virus was propagated in baby hamster kidney (BHK-21) cells, harvested and purified by sucrose gradient ultracentrifugation (Mecham *et al*., 1986). Whole virus preparations were treated with chymotrypsin to produce subcore and core particles. The cores were purified by caesium chloride gradient ultracentrifugation (Mertens *et al*., 1987). The purity of the core and whole virus preparations was determined by SDS–PAGE (Laemmli, 1970) and Coomassie blue or silver staining (Bio-Rad). The number of particles was calculated by measuring absorbance at 260 nm, where one A_{260} unit equals $3{\cdot}1 \times 10^{12}$ core particles or $3{\cdot}6 \times 10^{12}$ intact virus particles (Holmes *et al*., 1995).

■ **Cell lines.** KC cells from embryos of AK colony insects (Wechsler *et al*., 1991) were grown in Schneider's *Drosophila* medium supplemented with 6 mg/l reduced glutathione, 30 mg/l L-asparagine, 4·5 mM bovine insulin, 2 mM L-glutamine and 15% heat inactivated foetal bovine serum (FBS) at 30 °C. BHK-21, C6/36 and DM-2 cells were obtained from the ATCC. BHK-21 cells were grown at 37 °C in Eagle's minimum essential medium (MEM) plus 10% FBS. C6/36 cells, from *Aedes albopictus* larvae, were grown at 30 °C in Eagle's MEM with 10% heat inactivated FBS. DM-2 cells, from *D. melanogaster* embryos, were grown at room temperature (20–22 °C) in Schneider's *Drosophila* medium with 10% heat inactivated FBS. All the cell lines used support the growth of BTV; however, KC cells are more susceptible. No non-susceptible cell lines were available.

■ **Antibodies.** Anti-VP7 mouse monoclonal antibody (MAb 1AA4) was raised against BTV-11 and cross-reacts with VP7 of BTV-10 (Mecham *et al*., 1990). Polyclonal antisera to both BTV-10 and BTV-11 were produced in rabbits (Mecham *et al*., 1986). A polyclonal rabbit anti-idiotypic antibody (anti-Id), RAb2-A, was generated against a MAb (ATCC 1875) that is specific for an epitope on VP7 of BTV-10. This anti-Id recognizes an idiotope that is located within the antigen-binding site and is present on anti-VP7 from mice and cattle. Another polyclonal rabbit anti-Id, RAb2-B, was raised against an anti-VP7 MAb (ATCC 1877). This anti-Id recognizes an idiotope that is located outside the antigen-combining site (Zhou & Huang, 1995).

■ **Preparation of subcellular fractions.** Subcellular fractions were prepared from the insects and cell cultures described above. All cultured cells were washed with PBS (pH 7·4) prior to preparing subcellular fractions. Samples were homogenized in a loose-fitting Dounce homogenizer in sodium carbonate–sucrose buffer (1 mM $NaHCO_3$, 1·5 mM $MgCl_2$, 1·5 mM $CaCl_2$, 0·25 M sucrose, pH 8·0). Homogenates were sequentially centrifuged at 600 ***g*** for 10 min, 4000 ***g*** for 20 min, 10 000 ***g*** for 20 min and 105 000 ***g*** for 60 min. Buffers were pre-cooled and centrifugations were performed at 4 °C. The pellets of each centrifugation (P1, P2, P3 and P4, respectively) and the final supernatants (cytosol) were saved; pellets were suspended in water, and all samples were frozen at −80 °C until use. P1 contains unbroken cells, nuclei and large membrane fragments; P2 consists mainly of plasma membranes; P3 contains mostly mitochondria, lysosomes, peroxisomes, Golgi membranes and some smaller plasma membranes; P4 includes microsomal membranes, Golgi membranes and small fragments of plasma membranes; and the cytosol fraction contains the soluble components of the cytoplasm (Graham, 1984). Crude membrane preparations, which contained P2, P3 and P4, were also tested in ELISA binding assays and Western membrane overlay assays.

■ **Binding ELISA.** Subcellular preparations were diluted in 0·1 M carbonate buffer, pH 9·6, and 0·1 ml of the diluted sample was coated on the wells of a 96-well microtitre plate by overnight incubation at 4 °C. The wells were then treated with a blocking solution (3% BSA, 0·005% Tween 20, in PBS, pH 7·4) for 30 min and 0·1 ml of the purified virus preparation (ca. $1{\cdot}53 \times 10^9$ particles), which was diluted with the blocking solution, was added to each well and incubation continued for another 3 h. After washing with PBS containing 0·005% Tween 20 (TPBS), the amount of virus that bound to the subcellular components was quantified by addition of MAb 1AA4 and reaction with an anti-mouse antibody–peroxidase colour reaction system (BioGenex). Virus-free blanks were made in which TPBS was used in place of BTV samples, and rabbit pre-immune sera were tested for non-specific binding. Binding of the rabbit anti-Id to subcellular preparations coated on the microtitre plates was estimated by replacing the virus preparation with the anti-Id and directly reacting it with the anti-rabbit antibody–peroxidase colour reaction system.

■ **Western Blot**

(1) Membrane overlay assay. The proteins of purified whole virus (BTV-2 and BTV-10) were separated by SDS–PAGE (Laemmli, 1970) using a Mini-PROTEAN II apparatus (Bio-Rad) and transferred to nitrocellulose membranes. After incubation with the blocking solution and washing once with TPBS, the blots were incubated for 1 h at room temperature with either plasma membrane preparations (P2) or crude

membrane preparations of insects or cultured cells that were diluted to ca. 40 μg protein/ml with TPBS. The viral protein bands in the blots were visualized with MAb 1AA4 and a commercial anti-mouse IgG–alkaline phosphatase (AP) detection system, or with polyclonal anti-BTV and a commercial anti-rabbit IgG–AP detection system (Bio-Rad).

(2) Anti-Id overlay assay. P2 preparations from the AK colony, KC cells, C6/36 cells and DM-2 cells were electrophoresed, transblotted to nitrocellulose and blocked as described above. The blots were incubated with anti-Id RAb2-A or RAb2-B (0·25 to 1·25 μg/ml in TPBS) for 4 h at room temperature (ca. 22 °C). The anti-Id that bound to membrane protein bands was visualized with the anti-rabbit IgG–AP system. Molecular sizes of visualized bands were estimated according to their migration relative to pre-stained molecular size standards (Bio-Rad) that were loaded onto lanes in the same gel.

■ **Virus precipitation of proteins from plasma membranes.** Plasma membrane preparations were incubated overnight at 4 °C in TPBS with 0·5% (final concentration) Triton X-100. The solubilized proteins were collected from the supernatant following centrifugation at 105 000 ***g*** for 1 h. Core or whole virus preparations of BTV-10 were incubated overnight at 4 °C with blocking solution, pelleted by centrifugation at 105 000 ***g*** for 1 h and washed once with 2 ml of TPBS. Virus was then incubated with solubilized membrane proteins for 4 h at 4 °C with gentle stirring, pelleted, washed once with TPBS, and analysed by SDS–PAGE followed by silver staining.

■ **Protein assay.** Protein concentrations in the subcellular and virus preparations were determined with the Bradford reagent (Bradford, 1976) supplied by Bio-Rad. BSA was used as a standard.

Results

C. variipennis membranes reduce binding of VP7 to its antibodies

Viral proteins from purified virus were resolved by SDS–PAGE, transferred to nitrocellulose membranes and reacted with MAb 1AA4 and polyclonal anti-BTV. MAb 1AA4 detected only the VP7 band and polyclonal antisera detected all structural viral protein bands (Fig. 1). If the blot was incubated with P2 preparations of insects of the AK colony prior to reaction with MAb 1AA4, the intensity of VP7 staining was reduced (Fig. 1*c*, *d*). Pre-incubating the blot with crude membrane preparations or P2 of AK insects or KC cells also prevented the detection of the VP7 band (data not shown). When the blot was overlaid with adult *D. melanogaster* or larval *M. sexta* membrane preparations, the intensity of VP7 staining was not reduced (data not shown). Membrane preparations of the AK colony only slightly reduced the intensity of staining of the VP7 and some other viral protein bands by polyclonal anti-BTV (Fig. 1).

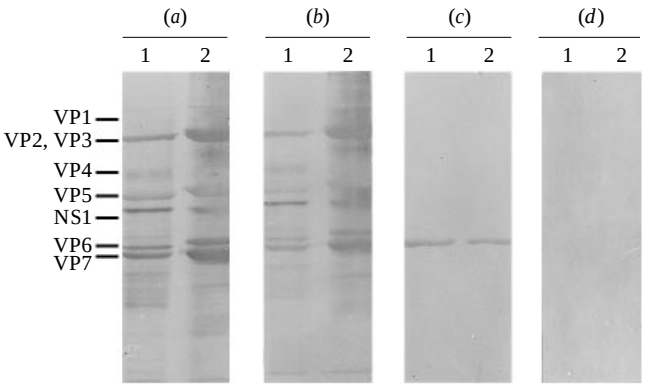

Fig. 1. Western analysis of the binding of BTV proteins with polyclonal anti-BTV (*a*, *b*) and monoclonal (*c*, *d*) anti-VP7 antibodies. BTV proteins were resolved on 8% SDS–polyacrylamide gels and transblotted to nitrocellulose membranes. The blots were pre-incubated with (*b*, *d*) or without (*a*, *c*) plasma membrane preparations from insect AK colonies before detection of BTV proteins with appropriate antibodies. Lane 1 contains BTV-10 and lane 2 contains BTV-11. Approximately $2{\cdot}8 \times 10^{11}$ particles were loaded in each lane.

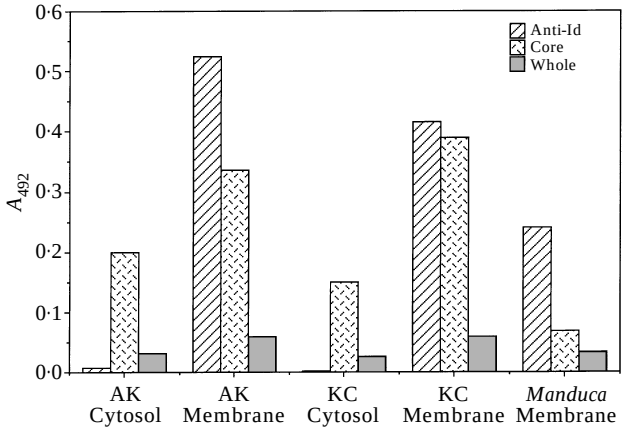

Fig. 2. Binding ELISA of VP7 anti-Id RAb2-A and BTV-10 whole and core particles to crude membrane and cytosol preparations. Values given are average readings of A_{492}/10 min, $n = 10$. The SD for each value was within 10%. The amount of subcellular preparation coated on the plates was ca. 2·5 μg per well and the amount of RAb2-A was 0·25 μg per well. Amounts of BTV-10 core and whole viruses were $1{\cdot}53 \times 10^9$ and $2{\cdot}89 \times 10^{10}$ particles per well, respectively.

Cores have higher binding affinity for the membrane preparations than intact BTV

Crude membrane fractions bound VP7 of both core and whole virus particles to a greater degree than cytosol fractions in the ELISA binding assay (Fig. 2). All membrane preparations (P1, P2, P3 and P4) had higher affinity than the cytosol for the core virus. P2 had the highest affinity for the core virus, followed in order by P1, P3 and P4, but the differences were not significant (data not shown). Core virus preparations gave higher absorbance readings than whole virus preparations for the membrane or cytosol fractions. Typically, the core preparations showed a sevenfold increase compared with whole virus preparations in binding KC crude membranes (Fig. 2). Therefore, core preparations were used to estimate binding kinetics. There was a hyperbolic correlation between BTV titres and the amount of virus bound to P2 preparations of AK

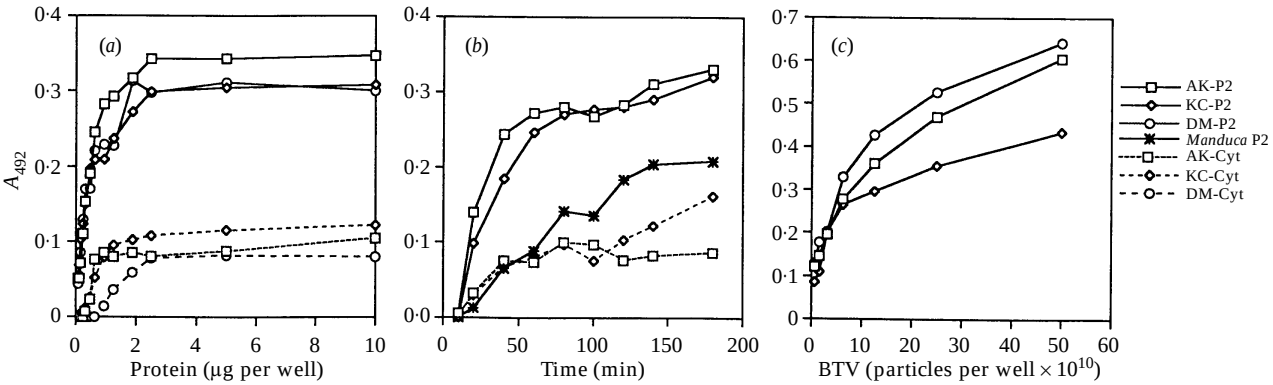

Fig. 3. Effects of membrane concentration (*a*), incubation time (*b*) and virus concentration (*c*) on the binding of BTV-10 cores (*a*, *b*) and whole particles (*c*) to plasma membrane and cytosol preparations. In (*b*) and (*c*), plates were coated with ca. 2·5 μg of subcellular preparation per well. In (*a*) and (*b*), BTV-10 cores were added to plates at a level of $1{\cdot}53 \times 10^9$ particles per well. In (*a*) and (*c*), BTV-10 particles were incubated with the subcellular preparation for 3 h.

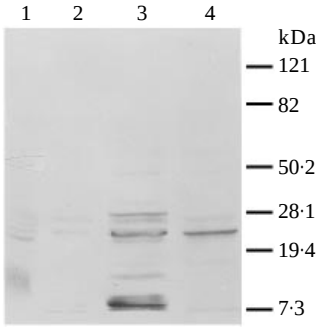

Fig. 4. Western analysis of the binding of VP7 anti-Id RAb2-A to resolved plasma membrane proteins. Plasma membrane proteins were resolved on 8% SDS–polyacrylamide gels and transblotted to nitrocellulose membranes. The blots were incubated with the anti-Id (1·25 μg/ml in TPBS). Bound anti-Id was visualized with an anti-rabbit IgG–AP system. Lanes 1, 2, 3 and 4 contained P2 preparations of DM-2 cells, C6/36 cells, KC cells and AK insects, respectively. Each lane received ca. 5 μg of protein.

insects, and KC and DM-2 cells (Fig. 3*c*). Growth of BTV was supported in both KC and DM-2 cells as well as AK insects. Binding of fixed amounts of cores correlated directly with the concentration of P2 adsorbed to the wells, until a plateau was reached (Fig. 3*a*). Similarly, the binding time-course for the cores to P2 from *C. variipennis* adults or KC cells reflected a saturable reaction, whereas non-vector *M. sexta* P2 gave a more linear pattern, indicating a higher probability of a non-specific reaction (Fig. 3*b*). The linear correlation coefficient (*r*) for the *M. sexta* P2 data was 0·89; for KC and AK, *r* values were about 0·60. The difference between the correlation coefficients was significant (Student's *t*-test, $n = 3$).

VP7 anti-Id binds to membrane preparations

The anti-Id RAb2-A bound to all membrane fractions, but not to the cytosol fractions in the ELISA binding assay (Fig. 2). As with the virus–membrane binding reaction, binding of the anti-Id to the membranes adsorbed to the wells correlated directly with the concentration of the anti-Id until a saturable plateau was reached (data not shown). In fact, at the concentrations used in the ELISA overlay assay, RAb2-A binding was more easily detected than binding of whole virus (Fig. 2).

VP7 anti-Id binds to specific AK and KC membrane proteins

In the anti-Id overlay assay, proteins in P2 preparations of the AK colony, KC cells, C6/36 cells and DM-2 cells were electrophoresed and transblotted to nitrocellulose membranes. The anti-Id RAb2-A was shown to bind strongly to a protein band with an apparent molecular mass of ca. 23 kDa that was present in the AK P2 preparation (Fig. 4, lane 4). A similar band was also present in the P2 preparation from KC cells. However, the anti-Id also reacted with several other proteins (lane 3). In contrast, there was a much lower binding of the anti-Id to the 23 kDa protein from C6/36 or DM-2 P2 preparations (lanes 1 and 2). Incubation of RAb2-A with anti-VP7 antibody ATCC 1875 prevented the binding of RAb2-A to the 23 kDa protein (data not shown). No band was visualized when RAb2-B or a pre-immune rabbit serum was tested in this Western analysis.

The 23 kDa protein binds to whole and core virus particles

After the plasma membranes were solubilized with Triton X-100 and incubated with core and whole virus, the 23 kDa protein bound to both core and whole virus particles. This protein was present in plasma membranes of AK insects and KC cells (Fig. 5*a*, lanes 6 and 7), and in the virus pellets after the virus was incubated with solubilized plasma membranes of AK insects and KC cells (Fig. 5*a*, lanes 2 and 3, *b*, lanes 3 and 4). After incubation with core and whole virus preparations, levels of this 23 kDa protein were greatly reduced in the solubilized

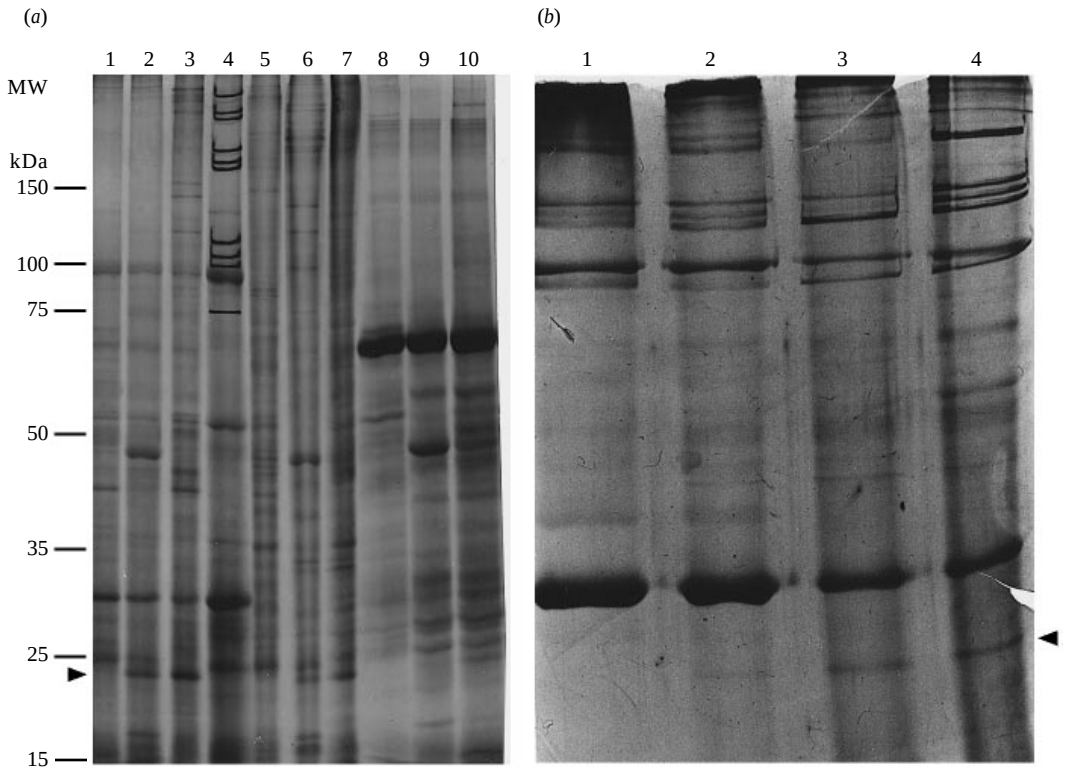

Fig. 5. SDS–PAGE analysis of precipitation of membrane proteins with BTV-10 particles. Whole or core BTV-10 particles were incubated with solubilized plasma membrane proteins. The BTV particles were then precipitated by centrifugation. The pellet and supernatant samples were resolved on 8% SDS–polyacrylamide gels and silver stained. (*a*) Samples were separated with a 14 × 16 cm SDS–polyacrylamide gel. Lanes 1, 2 and 3 contain pellets and lanes 8, 9 and 10 contain supernatants obtained by centrifugation of the incubation mixture of whole BTV-10 and solubilized plasma membrane proteins of C6/36 cells (lanes 1 and 8), AK insects (lanes 2 and 9) and KC cells (lanes 3 and 10). Lane 4 contains purified whole BTV-10. Lanes 5, 6 and 7 contain plasma membrane preparations of C6/36 cells, AK insects and KC cells, respectively. (*b*) Samples were separated with a 7·3 × 8 cm SDS–polyacrylamide gel. Lanes 2, 3 and 4 contain pellets obtained by centrifugation of the incubation mixture of core BTV-10 and solubilized plasma membrane proteins of C6/36 cells (lane 2), KC cells (lane 3) and AK insects (lane 4). Lane 1 contains purified core BTV-10. Note that RNA bands are visible in the lanes containing whole and core BTV-10.

plasma membranes of AK insects and KC cells (Fig. 5*a*, lanes 9 and 10). This protein was only slightly detectable in the plasma membrane preparations of C6/36 (Fig. 5*a*, lane 5, see also *b*, lane 2) or DM-2 cells (not shown). Pre-incubation of solubilized membranes with RAb2-A did not affect the binding of this 23 kDa protein to virus particles.

Discussion

The binding and penetration of a virus to a vector or host cell probably involves multiple interactions between viral and cellular membrane components. Our results suggest that VP7 is involved in binding of BTV to the membrane proteins of its vector, *C. variipennis*. VP7 is the surface protein of the core particle and is accessible at the outer surface of the whole virus particle (Huismans *et al.*, 1987*c*; Hyatt & Eaton, 1988; Grimes, 1995; Lewis & Grubman, 1990; Roy *et al.*, 1990). The observation that *C. variipennis* membrane preparations reduced detection of VP7 by MAb 1AA4 indicates that *C. variipennis* membrane preparations contain one or more components that block the binding site of VP7 for its MAb. The observation that membrane preparations of AK insects or the KC cells only slightly inhibited detection of VP7 by polyclonal antibodies suggests that only specific areas or regions of VP7 react with membrane proteins.

The binding of VP7 to membrane proteins was also shown in the ELISA binding assays and was apparent from virus precipitation of membrane proteins. The stronger reaction detected when core particles were used in the ELISA binding assay compared with that observed with whole virus can be explained by assuming that VP7 contains the binding epitope for membrane receptors. Virus core particles would have more VP7 exposed and bind membranes to a greater extent than whole viruses. This result could also be due to the fact that MAb 1AA4 is specific for VP7 (Mecham *et al.*, 1990). Since VP7 is more exposed in the cores, more MAb 1AA4 could bind to cores than to whole viruses. However, our data provide evidence that virus core particles bind to membrane preparations of vector insects even without the outer proteins VP2 and VP5. The core particles bound very well to membranes of

DM-2 cells. The DM-2 cells might have receptors for BTV, since this cell line is susceptible to BTV infection (data not shown).

Both core and whole particles bound to a 23 kDa protein that was present in the plasma membranes of AK insects or their cultured cells. There were a number of additional membrane proteins that bound weakly to the viruses and were easily washed away with TPBS (data not shown). The high affinity between the 23 kDa protein and the viral particles indicates that the binding is specific and makes this protein an excellent candidate for a receptor in *C. variipennis*. Our results suggest that both core and whole particles recognize and bind to the same membrane receptors. Since VP7 is exposed in the core and is not completely shielded in the whole virus, the receptor protein may attach only to VP7.

The fact that the VP7 anti-Id RAb2-A bound to a protein of a similar molecular size (23 kDa) to that bound by whole and core viruses supports the hypothesis that VP7 contains a binding epitope(s) for membrane receptors in vector insects. The anti-Id was made against an antigen-combining region of anti-BTV-10 VP7; therefore, it is an internal image that functionally mimics VP7 (Zhou & Huang, 1995). Because the 23 kDa protein exists predominantly in the plasma membrane of the vector insect or its cell culture with very little in the non-vector insect cell cultures, and because it binds to both core and whole virus, as well as to the VP7 anti-Id, it is likely a specific receptor that binds to VP7 of the virus. The finding that the VP7 anti-Id RAb2-A does not prevent binding of the 23 kDa protein to the core or whole virus particles suggests that this anti-Id may not bind to the same epitope. Alternatively, it may not have an affinity for the receptor that is high enough to compete with the biological form of VP7 in the BTV at the concentration of anti-Id used.

The importance of VP7 in BTV binding and infection of vector insects or cultured cells has been reported. Using intact BTV particles and cores, and infectious sub-viral particles (ISVP) obtained by treatment of whole particles with chymotrypsin, Mertens *et al*. (1987, 1989, 1996) found that the cores were as infectious to adults of *C. variipennis* and to cells derived from this insect (KC cells) as intact virus, but less infectious than ISVP. This was explained by assuming that the core binds to a different receptor(s) than does the whole particle or the ISVP, which contain partially degraded VP2 and VP5. Similarly, cleavage of VP2 by species-specific serum proteases in African horse sickness virus, another orbivirus vectored by *C. variipennis*, enhances infectivity of the virus in a laboratory colony of *Culicoides* (Marchi *et al*., 1995).

BTV must contend with the activity of midgut proteases after it is ingested by the insect. No information on *Culicoides* midgut protease activity is available, but chymotrypsin and other proteases have been found in the midguts of many haematophagous dipteran insects including mosquitoes, muscid flies and sandflies. It is reasonable to hypothesize that after BTV particles are ingested into the vector midgut from an infected host, the outer capsid proteins are cleaved by the midgut proteases. VP2 and VP5 would be partially degraded, and VP7 exposed to midgut peritropic membranes. VP7 would then initiate the specific binding of the virus to the membrane receptor(s), and the partially degraded VP2 and VP5 may facilitate entry of the virus into the cell. Evidence from both our laboratory (unpublished data) and others (Brookes *et al*., 1994) suggests that VP2 plays a role in virus penetration.

Our study provides further evidence that VP7 plays an important role in BTV infection of its insect vector and provides the first description of potential BTV receptor proteins from these insects. Further characterizaton of these proteins will lead to a mechanistic understanding of their interaction with VP7 and the potential importance of these proteins in vector competence. Similar studies have described a 32 kDa putative receptor protein for Venezuelan equine encephalitis virus in C6/36 plasma membranes (Ludwig *et al*., 1996). Further characterization of these receptors may provide insight into the infectious process of arboviruses.

Fifth instar larvae of *M. sexta* and adults of *D. melanogaster* were kindly provided by Lee Bulla and Nancy Petersen, respectively, both at the University of Wyoming, Laramie, Wyoming, USA.

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