Binding of MVL-2 Virus to A. laidlawii Cells

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Received January 4, 1983

Binding of MVL-2 virus, whose envelope lipids were radioactively labeled, to A. laidlawii JA1 cells was determined and characterized. The binding followed first-order kinetics and was temperature-dependent. All MVL-2 particles were capable of binding to A. laidlawii cells. At least 75 percent of radioactive MVL-2 bound represented specific binding which was markedly inhibited by EDTA. Virus infectivity was not essential for binding as inactivation of the virus by ultraviolet irradiation did not affect binding. Nevertheless, protein denaturing agents or proteolytic enzymes markedly inhibited MVL-2 binding, suggesting that the binding site of MVL-2 is of proteinaceous nature.

INTRODUCTION

The MVL-2 Acholeplasma viruses are unique among viruses of procaryotes in that they are spherical, envelope-bound viruses [1,2] that contain supercoiled, circular, double-stranded DNA [3]. The envelope of MVL-2 is composed of a lipid bilayer derived from the host cell membrane [4,5] and several proteins [4,6] of which at least two are localized on the external surface of the viral envelope [4].

Enveloped viruses, like other viruses, replicate by diverting the biosynthetic pathways of the host cells to virus production. To gain access to this machinery the viral genomes must enter the cell cytoplasm. Although little is known of the mechanism of such entry [7], it is apparent that it should include two major steps: (1) the binding of the virus through specific binding sites to their receptors on the host cell membrane, and (2) the penetration of the viral genome through the membrane barrier. The specific receptors for MVL-2 on the external surface of A. laidlawii cells were recently identified as the oligosaccharide chain of the lipoglycan molecules [8]. As glycoproteins were not detected in the MVL-2 envelope, the binding site is expected to be one of the proteins projected on the external surface. This binding protein would play a major role in recognition and attachment of the virus to the host cell. As a first step toward characterizing and defining this binding protein, we are devising an experimental system in which binding can be determined quantitatively under defined conditions.

MATERIALS AND METHODS

MVL-2 virus was grown on A. laidlawii JA1 cells and purified as previously described [4]. For labeling the virus 0.25 μCi/ml of [9,10-3H] oleic acid (60 Ci/mole) or 0.2 μCi/ml of [methyl-3H] thymidine was added to the growth medium. The purified virus was suspended in 0.1 M NaCl in 0.01 M Tris buffer pH 7.5 (TN buffer) to a concentration of ~1 × 10^{11} plaque-forming units/ml (PFU) and stored at −70°C until used.
Assessment of MVL-2 Binding

MVL-2 binding to *A. laidlawii* JA1 cells was assayed using an overnight *A. laidlawii* JA1 culture (*A*₆₄₀ = 0.25). The cells were harvested, washed once in 0.25 M NaCl, and resuspended to an absorbance of *A*₆₀₀ = 10 (∼5 × 10⁶ CFU/ml) in the reaction buffer containing 0.25 NaCl; 0.05 Tris; 0.01 M glucose; 0.0002 M CaCl₂; 2.5 mg/ml bovine serum albumin and DNase (10 μg/ml) adjusted to pH 7.5. To 0.6 ml samples of the cell suspension, aliquots of 0.2 ml freshly thawed labeled virus and 0.1 ml of the reaction buffer were added, and the mixtures were incubated for 15 minutes at 37°C. Controls in which *A. laidlawii* cells were replaced by 0.6 ml of the reaction buffer were included. After incubation, 0.4 ml samples in duplicate were pipetted on to the surface of silicone oil (0.2 ml) in 1.5 ml plastic microfuge tubes and centrifuged at 12,800 g for two minutes. Under these conditions, only virus bound to cells passed through the silicone oil, forming a pellet at the bottom of the tube. The unbound virus particles remained in the supernatant. After both the aqueous phase and the oil were removed by suction, the tip of the plastic tube (containing the virus-cell pellet) was cut off with a razor blade, placed in scintillation liquor, and counted. Binding results were expressed as ³⁵H-labeled virus bound (cpm) or as percentage binding obtained by determining the level of radioactivity in the pellet compared to that in the total reaction mixture.

RESULTS AND DISCUSSION

Kinetics of MVL-2 Binding to *A. laidlawii* Cells and the Effect of Temperature

Binding of MVL-2 to *A. laidlawii* cells was determined using ³⁵H-oleate labeled virus. The results were identical to those obtained with ³⁵H-thymidine labeled virus preparations, ruling out the possibility that the radioactivity found in the cell pellet of reaction mixtures containing ³⁵H-oleate labeled virus was due to the exchange of the ³⁵H-labeled lipid between the viral envelope and *A. laidlawii* membranes. Furthermore, in several experiments, the binding capacity determined from radioactivity measurements was found to be in good correlation with the number of plaque-forming units (PFU) bound. The number of bound PFU particles was calculated by subtracting the number of residual PFU left in the supernatant fluid after the cell-virus complexes had been pelleted from the total PFU in the reaction mixture.

The binding of MVL-2 to *A. laidlawii* cells follows first-order kinetics. Binding rates were rapid and temperature-dependent. Maximal binding at 23°C and 37°C was obtained after five minutes of incubation and did not change significantly on further incubation for up to 120 minutes at the same temperatures. Binding at 15°C and 4°C reached levels similar to those obtained at 37°C, but those levels were reached after 15 minutes and 45 minutes of incubation, respectively. Subsequent binding experiments were performed for 15 minutes at 37°C.

Binding Specificity of MVL-2

Figure 1 shows that the binding of ³⁵H-labeled MVL-2 virus was competitively inhibited by unlabeled virus. As 100-fold excess of unlabeled virus binding was inhibited by 75–80 percent, it seems that 75–80 percent of the labeled MVL-2 bound to *A. laidlawii* cells represents specific binding and 20–25 percent nonspecific binding. When the unlabeled virus was allowed to bind to *A. laidlawii* cells before the radioactive MVL-2 was added, binding of the labeled virus was more pronouncedly inhibited (insert, Fig. 1). Thus, when equal amounts (PFU/ml) of labeled and unlabeled virus were added simultaneously, the binding of ³⁵H-labeled virus was in-
FIG. 1. Competitive inhibition of $^3$H-labeled MVL-2 binding to *A. laidlawii* cells by unlabeled virus. Labeled virus ($1 \times 10^9$ PFU/ml) was added simultaneously with 0.1 to 100-fold excess of unlabeled virus, and binding was assayed as described in Materials and Methods. *Inset:* Time course of binding of $^3$H-labeled MVL-2 to *A. laidlawii* cells in the presence (closed symbols) or absence (open symbols) of equal amounts of unlabeled virus. The unlabeled virus was added together (○); 2.5 minutes before (▲); or 5 minutes before the labeled virus (■).

hindered by about 45 percent, whereas when the unlabeled virus was added 2.5 or 5 minutes before the addition of the labeled virus, 80 percent inhibition was observed.

**Binding as a Function of the Amount of MVL-2 Virus and *A. laidlawii* Cells**

Figure 2a shows that binding of MVL-2 to a constant amount of *A. laidlawii* cells ($2 \times 10^9$ CFU/ml) obeys first-order kinetics. The binding increased linearly with the increase in PFU in the binding mixture, reaching maximal levels at about $5 \times 10^{10}$ PFU/ml. The ratio of bound MVL-2 virus to *A. laidlawii* cells at the plateau was 1.5–2 (PFU/CFU). Hence, the amount of MVL-2 used in most binding assays was chosen to be within the linear region. When the amount of virus was kept constant ($2 \times 10^{10}$ PFU/ml) and the number of host cells (CFU) was varied, binding levels as
high as 98 percent were obtained (Fig. 2b), suggesting that practically all infective MVL-2 particles are capable of binding to A. laidlawii host cells.

**Effect of Divalent Cations on Binding**

The addition of Mg\(^{2+}\) or Ca\(^{2+}\) to the binding mixture at concentrations of up to 5 mM did not affect the binding of MVL-2 to A. laidlawii cells. Nevertheless, in the presence of 2 mM ethylenediaminetetraacetic acid (EDTA) the binding was inhibited by about 70 percent (Table 1), suggesting that the levels of divalent cations present within MVL-2 or A. laidlawii cells are sufficient to ensure optimal binding. The binding inhibition obtained by EDTA was completely restored by the addition of 5 mM of Ca\(^{2+}\), Mg\(^{2+}\), or Mn\(^{2+}\). The extent of MVL-2 binding in the presence of these cations was unaffected by the anions used (Cl\(^{-}\) vs. SO\(_4^{2-}\)). To rule out the possibility that the inhibitory effect of EDTA was due to the release of loosely bound component(s) from the viral surface or the A. laidlawii cell membrane, MVL-2 and A. laidlawii preparations were pretreated with EDTA for 30 minutes at 37°C. As is apparent from Table 2, in binding mixtures containing Mg\(^{2+}\), no significant differences were found in the binding capacity between pretreated and untreated preparations. The results strongly suggest that the inhibition obtained with EDTA is directly related to chelating of divalent cations required for binding.

**Binding of MVL-2 Exposed to Various Treatments**

Treating MVL-2 preparations by heating, ultraviolet irradiation, proteolytic enzymes, or urea resulted in a marked decrease in the infectivity of the preparations. Yet the effect of such treatments on the binding capacity of the virus varied (Table 2). Thus, after ultraviolet inactivation MVL-2 retained its capacity to bind to A. laidlawii cells, whereas inactivating MVL-2 by means which cause protein damage, such as heat or proteolytic enzymes, markedly inhibited the binding, apparently by damaging the binding sites. These results support the concept that the MVL-2 binding site(s) is of proteinaceous nature [9]. The high binding capacity retained by urea-treated virus preparations may suggest that the specific protein species removed by the urea treatment does not participate in the binding process. One cannot exclude, however, the possibility that the high binding levels obtained with urea-treated

| Preparations | EDTA and MgCl\(_2\) in Binding Mixture |
|--------------|-----------------------------------|
|              | EDTA (2 mM) | MgCl\(_2\) (5 mM) | MVL-2 Bound (% of Control) |
| Untreated    | -           | -               | 100                           |
| Untreated    | -           | +               | 98                            |
| Untreated    | +           | -               | 32                            |
| Untreated    | +           | +               | 94                            |
| Pretreated   | -           | +               | 96                            |
| with 2 mM EDTA | -           | +               | 92                            |
| Untreated    | Pretreated  | -               | 96                            |
| with 2 mM EDTA | +           | -               | 96                            |

| Pretreated   | Pretreated  | -               | 96                            |
| with 2 mM EDTA | with 2 mM EDTA | +               | 96                            |
TABLE 2
Binding Capacity of MVL-2 Virus Exposed to Various Treatments

| Treatment                      | Infectivity (PFU/ml) | MVL-2 Bound (% of Control)* |
|-------------------------------|----------------------|-----------------------------|
| None                          | 1.7 x 10^10          | 100.0                       |
| Heat (°C)                     |                      |                             |
| 45                            | 1.0 x 10^10          | 94.6                        |
| 65                            | 1.0 x 10^3           | 40.0                        |
| Ultraviolet Irradiation (minutes) |                  |                             |
| 1                             | 2.0 x 10^10          | 96.4                        |
| 10                            | 1.0 x 10^3           | 88.2                        |
| Proteolytic Enzymes (10 µg/ml) |                      |                             |
| Trypsin                       | 8.5 x 10^7           | 32.1                        |
| Chemotrypsin                  | ND                   | 23.4                        |
| Urea (7.5 M)                  | 3.0 x 10^6           | 92.8                        |

*MVL-2 virus suspended in TN buffer were treated either by heat (30 minutes at the temperatures shown), irradiated by an ultraviolet lamp (Mineralight V41 0.25A) at a distance of 20 cm for 1-10 minutes, treated with proteolytic enzymes for 30 minutes at 37°C, or with 7.5 M urea for 30 minutes at 4°C.

*Binding values of untreated cells served as controls. Of the control cell population, 34 percent bound to host A. laidlawii cells.

MVL-2 are in fact due to the formation of MVL-2 aggregates upon treatment. Such aggregates may bind only through a few viral particles, whereas the radioactivity level will be artificially high, representing all viral particles in the aggregate.

ACKNOWLEDGEMENT

This work was supported by a grant from the Israel Academy of Sciences and Humanities.

REFERENCES

1. Gourlay RN: Mycoplasmatales virus-laidlawii 2, a new virus isolated from Acholeplasma laidlawii. J Gen Virol 12:65–67, 1971
2. Gourlay RN, Garwes DJ, Bruce J, et al: Further studies on the morphology and composition of mycoplasmatales virus laidlawii 2. J Gen Virol 18:127–133, 1973
3. Nowak JA, Maniloff J: Physical characterization of the superhelical DNA genome of an enveloped mycoplasmavirus. J Virol 29:374–380, 1979
4. Greenberg N, Rottem S: Composition and molecular organization of lipids and proteins in the envelope of mycoplasmavirus MVL2. J Virol 32:717–726, 1979
5. Al-Shammari AJN, Smith P: Lipid composition of two mycoplasmaviruses, MV-Lg-L172 and MVL-2. J Gen Virol 54:455–458, 1981
6. Putzrath RM, Cadden SP, Maniloff J: Effect of cell membrane composition on the growth and composition of nonlytic enveloped mycoplasmavirus. Virology 106:162–167, 1980
7. Lenard J: Virus envelopes and plasma membranes. Ann Rev Biophys Bioeng 7:139–165, 1978
8. Al-Shammari AJN, Smith PF: Interaction of mycoplasma virus type 2 with cellular components of Acholeplasma laidlawii strain JAI. J Virol 36:120–124, 1980
9. Rottem S, Greenburg N: Molecular organization and selective solubilization of lipids and proteins in the envelope of mycoplasmavirus MVL-2. Rev Infect Dis 4:99–104, 1982