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NOTES

Mutant of Polyoma Virus with Impaired Adsorption to BHK Cells

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A mutant of polyoma virus PY235 has an impaired adsorption to guinea pig red blood cells and BHK-21 hamster cells. Adsorption to 3T3 mouse cells is much less inhibited. These altered adsorption properties are responsible for the apparent inability of PY235 to cause cell transformation or hemagglutination.

The assignment of viral gene functions which are responsible for the establishment and maintenance of cell transformation by oncogenic viruses has been attempted by several investigators. In the case of the DNA-containing polyoma (PY) virus, the search for viral mutants has mainly yielded mutants affected in the establishment but not in the maintenance of transformation (6, 8, 10). Only one mutant which is involved in the maintenance of some properties of cell transformation has been isolated (7, 9). Other mutants which are not capable of transformation cannot be analyzed for maintenance properties because of the lack of a conditional state (3, 4).

In this paper, we wish to describe the unusual properties of mutant PY 235, which was first believed to be a nontransforming mutant. Further analysis, however, revealed that the lack of transforming ability of PY 235 under standard conditions of infection was due to its adsorption properties. These peculiar adsorption characteristics are particularly evident in hamster cells and are also reflected in the abnormal hemagglutination (HA) given by this virus.

The general methods for titration and preparation of PY virus have been described (1, 2, 6). Infectivity was tested by plaque assay on 3T3 mouse cells (2, 6), and transformation of BHK-21 hamster cells was tested by the agar assay (12). HA was determined as described (1) after incubating guinea pig red blood cells (RBC) and virus at 4 C for 6 to 12 h in TD buffer (0.8% NaCl, 0.38% KCl, 0.01% Na_2HPO_4, and 0.3% Tris adjusted to the desired pH with 1 N HCl), pH 7.3 to 7.4, unless otherwise specified. The final concentration of RBC was 0.2%. All pH values given were normalized to 20 C.

Mutant PY 235 was isolated from a stock of large-plaque (LP) PY virus which had been mutagenized with hydroxylamine during a search for temperature-sensitive (TS) PY mutants (6). PY 235 was isolated as a TS mutant, because it produced normal plaques at 32 C, whereas at 39 C the efficiency of plating was reduced about 1,000-fold. At 37 C, the efficiency of plating was reduced about 10-fold with respect to 32 C, and the plaques had a fuzzy appearance (Table 1). The absence of plaque formation at 39 C was not, however, a consistent finding, as occasionally PY 235 gave fuzzy plaques also at 39 C with an efficiency about 50-fold lower than at 32 C. PY 235 was always propagated at 32 C.

Although PY 235 is somewhat TS in plaque formation, one-cycle growth experiments failed to reveal any temperature sensitivity of growth. Rather, the yield was reduced at any temperature to about one-tenth of that of wild-type (WT)-infected cells. This reduction of yield seems to be mainly due to a late defect, as synthesis of viral DNA is not impaired (Table 2). Accordingly, induction of PY-specific T-antigen in 3T3 cells was not significantly reduced with respect to WT-infected cells.

When PY 235 was tested for transforming ability on BHK-21 cells, it did not cause any detectable transformation even at a multiplicity of infection of 2,000 PFU/cell, irrespective of the temperature of incubation of the cells after infection (Table 3). This was also true when transformation of rat ReCL3 cells (3) rather than

that of hamster cells was measured. On the basis of this evidence, PY 235 appeared to have a late defect, causing a reduction of virus production and an apparent inability to transform in two different cell systems.

When PY 235 was tested for ability to agglutinate guinea pig RBC, it was found that under the standard conditions used in this assay (pH 7.2 to 7.4, 4 C) this virus was unable to cause any significant HA, even at concentrations which should have given between 1,000 and 10,000 HA units. That this was due to a lack of adsorption of the virus to RBC could be shown in experiments in which the binding of radioactively labeled PY 235 to RBC was measured (Fig. 1).

It is known that the two naturally occurring strains of PY virus, the small plaque (SP) and

TABLE 1. *Plaque formation by PY 235 at different temperatures*

Virus	PFU		
	39 C	37 C	32 C
WT	3.0×10^8	3.2×10^8	3.4×10^8
PY 235	$<1 \times 10^5$	3×10^{7a}	3×10^8

[a] "Fuzzy" plaques.

TABLE 2. *Growth of PY 235 at 32 C and 39 C in 3T3 cells*

Virus[a]	Yield (PFU/culture)		Viral DNA synthesis[b]	
	32 C	39 C	32 C	39 C
WT	7.5×10^7	1×10^8	20,000	18,000
PY 235	7×10^6	6×10^6	11,000	11,500

[a] Multiplicity of infection: WT, 10 PFU/cell; PY 235, 4 PFU/cell.

[b] Radioactivity (counts/min) incorporated into viral DNA in approximately 4×10^6 cells which were labeled from 20 to 32 h after infection at 39 C and from 26 to 46 h after infection at 32 C, respectively, in the presence of [^{3}H]thymidine (5 μCi, 0.7 μg/ml). Viral DNA was extracted by the method of Hirt (11) and analyzed by velocity sedimentation on neutral CsCl gradients.

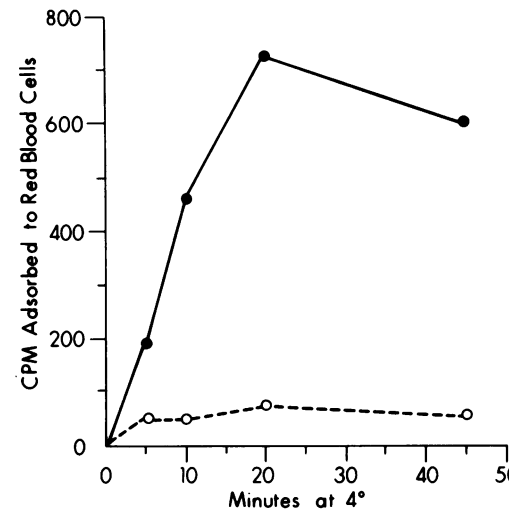

FIG. 1. *Adsorption of PY 235 to guinea pig RBC. PY 235 and WT, LP PY were labeled with [^{3}H]thymidine and purified. Specific activity was about 1 count per min per 10^3 PFU for both viruses. A 0.8% suspension of guinea pig RBC in TD buffer, pH 7.3, was mixed with an equal volume of virus suspension in TD. The final virus concentration was about 5×10^8 PFU, 5,000 counts per min per ml. Incubation was carried out at 4 C, with occasional shaking. At the times indicated, 0.4 ml-samples were withdrawn and diluted in TD. The RBC were centrifuged, resuspended in TD, and filtered through Whatman fiberglass filters. The filters were dried and counted in a Beckman scintillation spectrometer. A portion of the incubation mixture was spotted on filters to measure the total amount of counts per minute per sample, which corresponded to 2,100 counts/min for PY 235 and 2,200 counts/min for the WT virus.* ●, *WT virus;* ○, *PY 235.*

TABLE 3. *Transformation of BHK cells by PY 235 under different conditions of virus adsorption*

Expt	Adsorption	No. of transformed colonies in 10^5 cells[a]			
		WT LP virus[b]	Ratio (transforming units-PFU)	PY 235 virus[b]	Ratio (transforming units-PFU)
I	pH 7.3, 36.5 C	1,200	6×10^{-6}	0	$<1 \times 10^{-8}$
II	pH 7.3, 36.5 C	94	5×10^{-6}	0	$<1 \times 10^{-7}$
	pH 7.3, 24 C	650	3.2×10^{-5}	4	4×10^{-7}
	pH 6.5, 24 C	520	2.6×10^{-5}	95	1×10^{-5}

[a] Determined by the agar assay; colonies were counted after 10 days of incubation at 37 C.

[b] Multiplicity of infection: experiment I, 2,000 PFU/cell; experiment II: WT, 200 PFU/cell; PY 235, 100 PFU/cell.

LP, have a different spectrum of HA (5). Figure 2 shows the pattern of HA by SP and LP PY when the assay is performed at different pH's at 4 C; also in Fig. 2 is the same analysis for PY 235. It can be seen that HA was almost completely inhibited at pH above 7.2, whereas at pH 6.5 the virus was capable of HA. At this pH, both WT strains were slightly inhibited.

We then tested the adsorption of ^{3}H-labeled WT and 235 PY virus to 3T3 and BHK cells. Adsorption was determined at 36 C, pH 7.3, in suspension (Fig. 3). It can be seen that the adsorption of PY 235 to mouse cells is slow and reaches only about 40% of the WT values. Adsorption to BHK cells is much more inhibited, reaching, at most, 5% of the WT. Because this type of experiment generally gives a rather high background, it is likely that the adsorption of PY 235 to BHK under these conditions is close to zero. It is interesting that the adsorption to 3T3 is inhibited only to about one-half of the WT. This is in line with the finding that the efficiency of productive infection of 3T3 cells by PY 235 is not very different from that of WT PY. The fact that under standard conditions PY 235 adsorbs to 3T3 cells much more efficiently than to BHK cells suggests that the receptors for PY virus of the two cell types are not the same. Alternative interpretations of this fact are possible, however.

These experiments suggested that the lack of

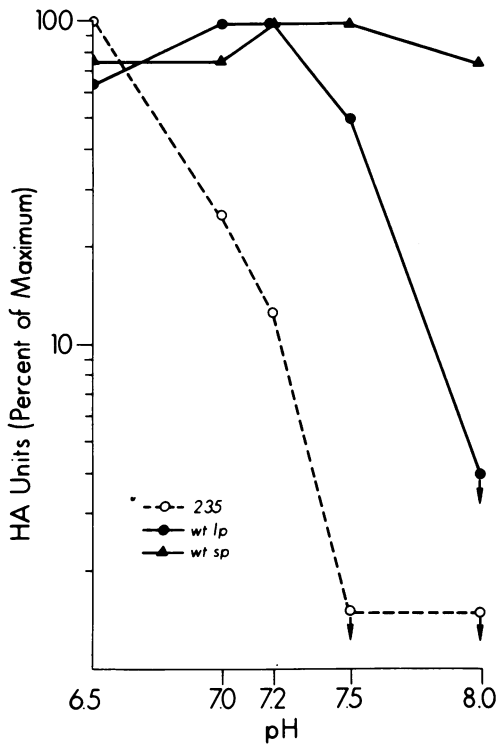

FIG. 2. *HA of PY 235 virus, LP and SP WT virus at different pH's. Guinea pig RBC were suspended in TD buffer of the desired pH at a concentration of 0.4%. A 0.4-ml amount of RBC suspension was mixed with 0.4 ml of the virus dilutions in TD buffer of the same pH and incubated at 4 C for 6 h. Data are expressed in percentage of maximum agglutination, which corresponded to 512 HA units for PY 235 and 256 HA units for both LP and SP WT virus.*

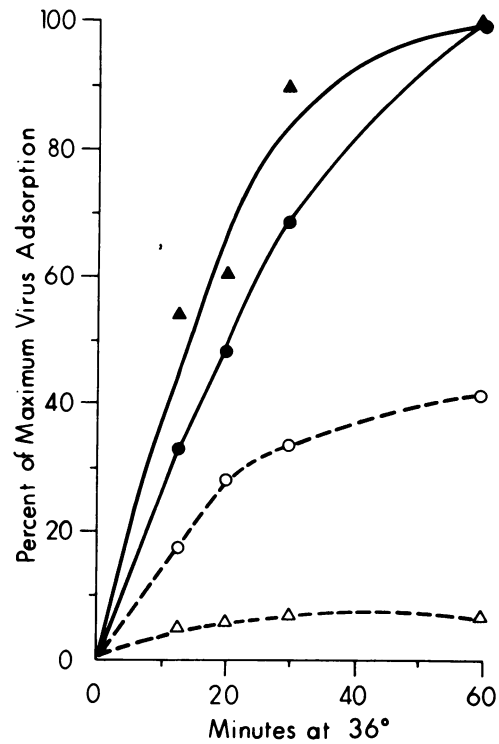

FIG. 3. *Adsorption of PY 235 to BHK and 3T3 cells. BHK and 3T3 cells were trypsinized, resuspended in TD buffer, pH 7.3, and incubated at 36 C in the presence of WT LP or PY 235, which had been labeled with [^{3}H]thymidine. The final concentration of cells was 1 × 10^6/ml, and the virus was 8,000 counts/min, 8 × 10^6 PFU/ml. Incubation was carried out at 36 C, keeping the mixture in constant agitation by using a magnetic stirrer. At the times indicated, 0.5-ml samples were withdrawn and the cells were washed three times by centrifugation, precipitated with trichloroacetic acid, and counted. Data are expressed as percentage of the maximum adsorption of the WT virus, which corresponded for BHK to about 25% of the input and for 3T3 to about 20%. ▲, BHK, WT polyoma; ●, 3T3, WT polyoma; △, BHK, PY 235; ○, 3T3, PY 235.*

transforming ability of PY 235 was due to defective adsorption to BHK under the standard conditions of infection. To test this hypothesis, transformation assays were carried out by infecting cells in suspension at various pH's and temperatures, all procedures thereafter being the same. Some of the results are shown in Table 3. It can be seen that WT, LP PY virus has the best transformation efficiency when the adsorption is carried out at pH 7.3 at room temperature. At lower pH, the efficiency of transformation is slightly lower, and it is almost 10-fold lower when the adsorption is carried out at neutral pH at 37 C. (SP PY virus has about the same efficiency over a wide range of temperatures and pH's of adsorption.) The results obtained with PY 235 confirmed the hypothesis that its inability to transform was due to a lack of adsorption. No transformation is observed when adsorption is carried out at 37 C, pH 7.3. When adsorption is carried out at 24 C, pH 7.3, some rare transformants are produced, and their number increases more than 20-fold when the adsorption takes place at pH 6.5. Under these conditions, the efficiency of transformation (transforming units/PFU) is still about three-fold lower than the WT, but it is likely that this small difference is still due to inefficient adsorption. BHK cells transformed by PY 235 displayed the typical morphology of PY-transformed BHK and were not TS for growth in agar medium.

Thus, the lack of transformation observed with PY 235 is probably only due to lack of adsorption to BHK cells. The properties of this mutant affect the adsorption to RBC and BHK cells much more than the adsorption to mouse cells. As mentioned before, this suggests that the PY receptors of the two cell types are different.

The partial temperature sensitivity of PY 235 plaque formation can also probably be explained by impaired adsorption and reinfection of neighboring cells at 39 C.

It is conceivable that the alteration of the coat proteins of PY 235, which must be responsible for its defective adsorption, might also play a more important role in the establishment of transformation. However, a change in the conditions of adsorption alone restores the transforming ability of PY 235. PY 235 virus, incubated at pH 6.0 for 2 h and then adsorbed at pH 7.3, is still incapable of HA or of transformation. It is very likely that the defect of PY 235 is caused by a mutation in a coat-protein gene. This mutation, which renders the virus defective in adsorption, causes, in addition, a low efficiency of packaging. In view of the finding that adsorption of PY 235 is much more inhibited in nonpermissive than in permissive cells, careful controls of effective penetration should be performed before any PY mutant can be considered nontransforming.

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