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MULTIPLICITY REACTIVATION WITH REOVIRUSES

A. HOSSAIN

Department of Microbiology, Faculty of Medicine, Riyad University, Riyad, Saud Arabia

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Summary. — Type 1 and type 3 reoviruses were found to be inactivated by UV radiation with single-hit kinetics. To get a measure of the extent of multiplicity reactivation in this system, the standard phage technique of plating cells as infectious centres after adsorption of the irradiated virus was used. The results obtained showed that both intra-typic and inter-typic multiplicity reactivation occur with reoviruses.

Key words: reovirus; multiplicity reactivation

Introduction

Human reovirus has a double-stranded RNA genome consisting of ten segments. Three serotypes exist. Each of the ten segments is considered to be a single gene (Cross and Fields, 1977). It has been shown that the mechanism of recombination in the case of reoviruses is by reassortment of genes (Hossain and Graham 1978*a*, *b*). Also marker-rescue has been found to occur between reovirus wild-type and mutants (Hossain and Graham, 1981) The present paper deals with the occurrence of both intra- and inter-typic multiplicity reactivation (MR) with UV-inactivated type 1 and type 3 reoviruses.

Materials and Methods

Cells and virus. L cells were grown in suspension or as monolayer cultures in Eagle's minimal essential medium (MEM) supplemented with 5% foetal calf serum. The Dearing strain of reovirus serotype 3, previously termed R^3 and the Lang strain of reovirus serotype 1 were used. Each reovirus strain was plaque purified, grown up into a large lysate and the virus then purified for use in the various experiments. Virus was assayed by the standard plaque method or by infectious centre procedure as decribed (Hossain and Graham, 1978*a*).

UV inactivation of virus. Samples of purified virus in phosphate-buffered saline (PBS) were sonicated for three 30-second intervals in the cold to break up clumps, and then inactivated with UV radiation as described (Hossain and Graham, 1978*a*).

Results

MR with reovirus

Type 1 and type 3 reoviruses were inactivated by UV radiation with similar single-hit kinetics. To get a measure of the extent of MR in this

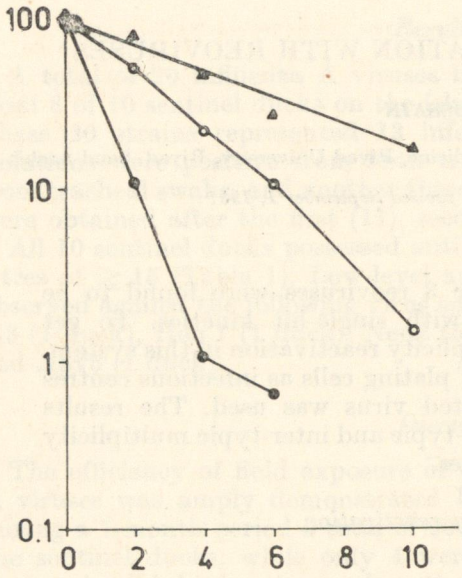

Fig. 1.
Multiplicity reactivation of UV-irradiated type 3 reovirus
Purified virus was irradiated for various periods of time (abscissa; time in min). After each interval cells were infected with different MOI of the irradiated virus and plated as infectious centres. ● = 0.01, ○ = 6 and ▲ = 12 refer to the MOIs had the virus not been irradiated.
Ordinate: virus survival (%)

system, we used the standard phage technique of plating cells as infectious centres after adsorption of the irradiated virus. A purified preparation of type 3 virus was sonicated and exposed to UV radiation for various periods of time. After each UV dose, cells were infected with the irradiated virus at several multiplicities of infection (MOI) and plated as infectious centres to determine the kinetics of viral inactivation at each MOI.

As shown in Fig. 1, at MOI = 0.01 PFU/cell the inactivation curve of virus was single-hit to a survival of approximately 10^{-2}. At a MOI of 6 or 12, however, at any given dose, the survival of infectious centres was much higher than expected; the higher the MOI, the greater the fraction of surviving infectious centres. Reovirus thus follows the classical picture of MR, and the increased survival of infectious centres at higher MOI would be explained as the increased chance of unhit genomic segments reassorting to form complete infectious genomes.

Intertypic MR with reovirus

If intertypic MR could occur with reoviruses, it might be a useful means to form intertypic recombinats. Experiments were set up to determine the extent of intertypic MR of irradiated type 1 and type 3 virus under different conditions and the results are shown in Table 1.

Taking one of the experiments as an example, cells were infected with irradiated type 1 virus at MOI =2 and 4 PFU/cell or with irradiated type 3 virus at MOI =2 and 4 PFU/cell or mixedly infected with the two irradiated viruses each at MOI =2 PFU/cell. In each case the cells were plated as in-

Table 1. Intertypic and intratypic multiplicity reactivation of UV-irradiated reovirus types 1 and 3

Inactivated virus type	MOI	Infectious centres per 1000 cells Found	Expected*
1	0.1	9	1
3	0.1	12	1
1 + 3	0.1 + 0.1	38	2
1	0.2	31	2
1	0.2	31	2
3	0.2	24	2
1	2	33	20
3	2	33	20
1 + 3	2 + 2	82	39
1	4	73	39
3	4	61	39
1	3	64	30
3	3	60	30
1 + 3	3 + 3	168	58
1	6	127	58
3	6	112	58

* Calculated on the actual MOI which was approximately 10^{-2} of the MOI shown in column 2. Suspensions of purified type 1 and type 3 reoviruses were sonicated and irradiated for 6 min, to approximately 10^{-2} survival. Monolayers of L cells (approx. 2×10^6 cells/dish) were infected with one or both of the irradiated viruses at the MOI shown in column 2. The MOI here was calculated based on the titre of the unirradiated virus, the actual being 10^{-2} of the stated MOI. The cells from each monolayer were suspended with trypsin and a known number of cells were plated as infectious centres with the results shown in column 3.

fectious centres. The number of infectious centres found always exceeded the expected number showing that MR had occurred. In particular, the mixed infection with type 1 and 3 viruses gave a significant increase in infectious centres over the sum of the two infections at a MOI =2 PFU/cell and approximately the same number of infectious centres as found with the two viruses separately at a MOI =4 PFU/cell. This suggests that the extent of MR between the viral types was approximately the same as that with each type alone and this conclusion is supported by the other two sets of data in Table 1. In this type of experiment the fraction of infectious centres derived from intertypic recombinants was 0.25 of the total infectious centres.

Discussion

It has been shown that the mechanism of recombination in the case of reoviruses is by reassortment of genes (Hossain and Graham, 1978*a*, *b*) and that cross reactivation can also occur between reovirus wild type and mutants (Hossain and Graham, 1981). The present study dealt with another aspect of the recombination between reoviruses. It showed that UV-irradiated reovirus followed single-hit kinetics. Also, reovirus exhibited the classical picture of MR; the higher the MOI, the greater the proportion of surving infectious centres. This phenomenon can be explained by the fact that at

higher MOI there is an increased chance of unhit segments reassorting to form complete infectious genomes.

The major outcome of this work was the demonstration that besides the formation of intratypic recombinants, recombinants between two serotypes (intertypic recombinants) occur after UV irradiation. The frequency of such recombinants was 25%. These intertypic recombinants would be very useful in various studies on reovirus genetics, in particular in the mapping of the reovirus genome and assignment of polypeptides produced to the genomic segments.

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