Plaque Formation with Simian Virus 40: Enhancement by Dimethyl Sulfoxide

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Dimethyl sulfoxide (DMSO) added to agar overlays during plaque assays of simian virus 40 (SV40) in CV1 monkey cells increases the plaque size and number and enables plaques to be read several days earlier than usual. DMSO appears to act during development of plaques, perhaps by causing cell lysis at smaller burst sizes in the presence of near-lethal DMSO concentrations. It does not act synergistically in determining virus inactivation with UV light and is equally effective on wild type and a late mutant of SV40.

Plaque formation by polyoma virus can be enhanced by adding dexamethasone to the agar overlay (3) and transformation can be enhanced by adding dimethyl sulfoxide (DMSO) (2). Experiments were done to determine whether DMSO could be similarly used for improving plaque formation with simian virus 40 (SV40).

Wild-type SV40, large plaque strain SP-1, was a gift from D. Lindstrom and a temperature-sensitive late mutant of SV40, Ts 661, was a gift from G. Kimura (1). Virus stocks were prepared by infecting monolayers of CV1 monkey cells with low multiplicities of SV40 (less than 0.1 PFU per cell). After extensive cell vacuolation had occurred, the medium and cells were harvested and crude viral extracts were prepared by sonic treatment of the cell suspension. Cell debris was removed by low-speed centrifugation and the supernatant was used for subsequent infections without further purification.

For plaque assays, confluent monolayers of CV1 cells were infected with 0.2 ml of appropriate dilutions of virus stocks in phosphate-buffered saline (PBS). Adsorption of virus was allowed to occur at 37 C (wild type) or 32 C (Ts 661) for 2 h with frequent agitation. At the end of the adsorption period, monolayers were overlaid with 10 ml of Eagle minimum essential medium containing 0.9% agar and grown at 37 C (wild type) or 32 C (Ts 661) for periods between 10 and 20 days. Agar-covered monolayers were stained by adding 3 ml of agar medium containing 0.01% neutral red and incubating overnight; plaques were scored by transmitted light.

In one set of experiments, 0.5 ml of virus stocks was spread in a thin layer in petri dishes and irradiated with 254-nm UV light at an

incident dose rate of approximately 60 ergs/mm²/s (calibrated with a Yellow Springs Instrument model 65 radiometer). After irradiation, virus dilutions were made and cells were infected as for plaque assays.

Two methods were used to determine the effects of various concentrations of DMSO on plaque formation. In one, various concentrations of DMSO in 0.1 ml of PBS were added to cell monolayers at the end of the adsorption period, immediately (i.e., 1 to 2 min) before agar was added. In the other, DMSO was dissolved in agar before covering the monolayers.

Both methods increased plaque numbers at DMSO concentrations between 0.1 and 0.5% (Fig. 1) and plaque size (Fig. 2). The first procedure caused a greater increase in number, which may be because monolayers were only transiently exposed to a high concentration of DMSO that would be lethal if maintained for long periods but was tolerated during the brief period before agar was added and the concentration thus decreased 100-fold. Concentrations above 1% were lethal for the cells, although plaques were occasionally detectable at 1%. If infected monolayers were incubated for 24 h before agar and DMSO were added, DMSO still increased plaque number, but less than when it was added earlier (Table 1).

The main effect of DMSO appears to be to increase plaque size so that very small plaques become visible earlier. Extending the incubation time at 37 C from 11 to 18 days minimized the effect of DMSO on plaque number, thus giving sufficient time for small plaques to become visible at all concentrations of DMSO (Fig. 2).

These results suggest that the speed and

FIG. 1. (A) Number of plaques formed by wild-type SV40 grown in CV1 cells after various concentrations of DMSO were added in 0.1 ml of PBS immediately before 10 ml of agar was added to the monolayer. DMSO concentration shown is the final dilution in the 10 ml of agar. Symbols: ●, assayed 11 days postinfection; ○, assayed 18 days postinfection. All data points are the mean of two or more dishes except for those 18 days postinfection, which are single dishes. (B) Number of plaques formed by wild type SV40 grown in CV1 cells with various concentrations

precision of SV40 plaque assays can be increased by adding DMSO. But if DMSO were synergistic with other treatments or had different effects on various SV40 mutants, its use would bias results. These possibilities were checked by determining the effect of DMSO on UV inactivation of SV40 wild type and the Ts 661 late mutant (Table 2). DMSO increased the plaque numbers for both wild-type and Ts 661 virus but did not significantly alter the surviving fraction of either after irradiation. Thus, the drug did not bias the results obtained from the radiation experiments.

In these experiments, then, DMSO added to agar overlays improved the speed and precision of plaque assays with SV40. The improvement is not so great as that afforded by dexamethasone on polyoma virus plaque formation (3), but it appears sufficient to be experimentally useful. For studies of UV inactivation of wild type and a late mutant, DMSO had no apparent synergism with radiation and is therefore a satisfactory additive. In other studies possible synergistic effects should be checked before DMSO is used extensively.

The mechanism of DMSO action does not appear to be limited to the initial stage of virus adsorption (Table 1) and it would seem that the drug simply increases the rate of growth of plaque size. This could occur if cells are lysed

of DMSO dissolved in the agar before covering cell monolayers. Symbols: ●, assayed 11 days postinfection; ○, assayed 13 days postinfection. All data points are the mean of two dishes.

FIG. 2. Photograph of CV1 monolayers infected with identical portions of wild-type SV40 and grown for 12 days at 37 C. (A) No additive. (B) 0.5% DMSO dissolved in the agar before covering the cell monolayer.

TABLE 1. *Number of wild-type SV40 plaques scored at 11 days postinfection and the influence of DMSO added before the agar overlay^a*

Expt	Plaque no./50-mm dish		
	No DMSO	DMSO added postinfection	DMSO added 24 h postinfection ^b
Unirradiated SV40 (10^{-6} dilution)	13	49	24
	14	41	13
	6	43	
	13		
	16		
	Mean = 12.4	Mean = 44.3	Mean = 18.5
4-min UV-irradiated SV40 (10^{-5} dilution)	8	22	22
	10	26	17
	Mean = 9	Mean = 24	Mean = 19.5

^a DMSO in 0.1 ml PBS was added to cell monolayers just before agar was added; final concentration of DMSO in the agar was 1%.

^b Cell monolayers grown in Eagle's medium plus 10% calf serum for 24 h before addition of DMSO and agar.

TABLE 2. *Plaque numbers of control and UV-irradiated SV40 after addition of 0.1 ml of DMSO in PBS immediately before addition of agar overlay*

Expt	Concn of DMSO in agar overlay				
	0%	0.001%	0.01%	0.1%	1.0%
Control wild-type virus ^a (10^{-6} dilution)	13	11	13	56	
	14	12	16	46	
	16		26	43	
	6				
	13				
Wild type after 4-min UV irradiation ^a (10^{-5} dilution)	8	8	9	25	
	4		8	12	
	8		13	22	
Fractional survival UV/control	0.050	0.069	0.054	0.041	
Control Ts 661 ^b (10^{-7} dilution)	2				10
	6				17
Ts 661 after 7-min UV irradiation ^b (10^{-5} dilution)					19
	6				32
	9				25
Fractional survival UV/control	0.025				0.018

^a Scored 13 days postinfection.

^b Scored 20 days postinfection.

with smaller burst sizes in the presence of DMSO, since enhancement of plaque formation occurred at concentrations just below those at which DMSO was lethal (Fig. 1). The enhancement effects of DMSO on SV40 plaque formation may therefore not be unique to this drug; similar effects might occur when plaque assays are done with any of a number of drugs at near-lethal concentrations. Preliminary results indicate that millimolar concentrations of caffeine also can increase plaque sizes of SV40

(unpublished observation), but since caffeine acts synergistically with UV light in viral inactivation (4), its use merely to enhance plaque size could bias some experimental results.

In conclusion, DMSO seems to make it possible to do plaque assays with SV40 more rapidly. At 37 C reliable assays are practicable in 11 days or less in CV1 cells, and if this effect is applicable to other cell types it might provide a method for increasing the speed and convenience of SV40 plaque assays.

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