Effect of Oxidized Spermine and Other Aldehydes on the Infectivity of Vaccinia Virus

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Received for publication 16 August 1971

The antiviral activity of oxidized spermine was compared with that of other aldehydes. Suspensions of vaccinia virus were incubated at 37 C with various concentrations of the aldehydes, and the infectivity of the viruses was determined by the plaque assay. Oxidized spermine at a concentration of 0.82 mM completely inactivated a suspension of vaccinia virus (1.4 x 10^3 plaque-forming units) after incubation at 37 C for 10 hr. Glutaraldehyde and formaldehyde were less active when compared on a molar basis, but acrolein resembled oxidized spermine in its antiviral activity. Because acrolein is produced from oxidized spermine at only 20 to 30% yield, it is unlikely that the biological activity of the latter is due to acrolein formed during the spontaneous degradation of oxidized spermine.

Numerous studies have demonstrated the toxicity of aldehydes for various viruses. Formaldehyde is commonly used for the preparation of viral vaccines because of its ability to impair the infectivity but not immunological properties of viruses. Glutaraldehyde is also an antiviral agent and has been used to inactivate yellow fever virus (11) and myxoviruses (4, 5, 17). Myxoviruses may also be inactivated by another aldehyde, oxidized spermine (3-5, 12, 14), obtained by the enzymatic oxidation of the polyamine spermine [NH(CH2)3NH(CH2)3NH(CH2)3NH2]. Oxidized spermine, which was identified as

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is unstable at alkaline pH and gives rise to high-molecular-weight condensation products (5, 13) and to acrolein as a result of a β-elimination reaction (1, 2, 13; Tabor et al., Fed. Proc. 23:385). In this study, we compared the antiviral activity of formaldehyde, glutaraldehyde, and acrolein with that of oxidized spermine. It is shown that oxidized spermine is more potent than formaldehyde and glutaraldehyde in inactivating vaccinia viruses.

MATERIALS AND METHODS

Chemicals. Spermine tetrahydrochloride was obtained from Fluka AG, Buchs, Switzerland; glutaraldehyde (25%) was supplied by K and K Laboratories, Plainview, N.Y.; acrolein (stabilized by hydroquinone) was the product of BDH Chemicals Ltd, Poole, England, and formaldehyde (36%), of Agan Chemical Manufacturers, Tel Aviv, Israel. Catalase was the product of Boehringer and Soehne, Mannheim, W. Germany. Serum amine oxidase was prepared according to Tabor, Tabor, and Rosenthal (19), with omission of the last calcium phosphate gel step. The preparation was purified 70-fold to a specific activity of 50 spectrophotometric units/mg (19). The oxidation of spermine was carried out in a Warburg flask containing 0.2 ml of amine oxidase (100 units), 1.0 ml of 0.2 M tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.0), 0.1 ml of catalase (750 units), 1.2 ml of physiological saline (NaCl, 0.85%), and 0.5 ml of spermine (10 μmoles).

Viruses and biological materials. The WR strain of vaccinia virus (obtained from the Department of Virology of this Institute) was titrated on 2-day confluent monolayers of monkey kidney cells (BSC). Cell cultures were propagated in Eagle's medium containing 10% heat-inactivated calf serum. Virus (0.2 ml), appropriately diluted in phosphate-buffered saline (PBS), was inoculated onto washed cultures, adsorbed for 45 min at 37 C, and then supplied with 2 ml of Eagle's medium containing 2% heat-inactivated calf serum. Cultures were incubated for 48 hr at 37 C and then were stained with 0.1% crystal violet in 0.1 M citric acid supplemented with 1% Formalin, to reveal plaques. Inactivation was carried out at 37 C, by shaking viral suspensions for desired times with equal volumes of inactivating agents (or nutrient broth in the control experiments).

RESULTS

Inactivation of vaccinia virus by oxidized spermine. Preliminary experiments indicated that oxidized spermine (0.5 mM, final concentration) caused a definite reduction in the in-
fectivity of vaccinia virus after exposure at 37 C for 3 hr (12). It was therefore of interest to study the effect of oxidized spermine on the infectivity of the virus as a function of the aldehyde concentration. Suspensions of viruses were intermittently shaken, for 24 hr at 37 C, with oxidized spermine diluted in nutrient broth. Figure 1 shows that oxidized spermine at a concentration greater than 0.8 mM (final concentration) caused a reduction in infectivity of at least 7 log units so that no plaques were detectable. Lower concentrations of the drug caused partial inactivation. Kinetic studies showed that the number of infective viruses decreased exponentially as a function of exposure time (Fig. 2). It is also evident that no plaques were formed after incubation of 1.4 x 10⁶ plaque-forming units (PFU) with 0.82 mM oxidized spermine for 10 hr (Fig. 2).

In the above experiments, partially purified serum amine oxidase was used. This is not essential, since crude heat-inactivated calf serum contains amine oxidase activity (65 units/ml; 62 mg of protein per ml). Therefore, incuba
ting vaccinia virus (1.7 x 10⁷ PFU/ml) with spermine (0.55 mM) in the presence of heat-inactivated calf serum (50% solution in nutrient broth) for 6 hr at 37 C resulted in a significant decrease in infectivity (a titer of 8.2 x 10⁴ PFU/ml). The titer of the control (which contained only heat-inactivated calf serum) was 5.5 x 10⁷ PFU/ml. The extent of inactivation was even more pronounced when 1.1 mM spermine was used (infective titer, 6 x 10² PFU/ml) or when incubation was continued for longer periods.

**Inactivation of vaccinia by other aldehydes.** The following experiments were designed to find out whether other mono- or di-aldehydes were as effective in inactivating vaccinia virus as oxidized spermine. Formaldehyde at a concentration of 3 mM had only a moderate effect on the infectivity of the virus, and 1.5 mM formaldehyde reduced the infectivity by approximately 1 log unit after incubation for 5 hr at 37 C (Fig. 3). It should be noted that oxidized spermine at a much lower

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**Fig. 1.** Inactivation of vaccinia viruses by oxidized spermine at various concentrations. Viruses were incubated with the drug at 37 C for 24 hr, and infectivity was determined by the plaque assay method.

**Fig. 2.** Effect of oxidized spermine on the infectivity of vaccinia virus. Suspensions of the virus were incubated with the drug for the times indicated, and infectivity was determined by the plaque assay method.

**Fig. 3.** Effect of formaldehyde on the infectivity of vaccinia virus.
concentration (0.41 mM) caused a similar inactivation (cf. Fig. 2). It could be argued that oxidized spermine is more active than formaldehyde because it is a dialdehyde. We therefore compared the antiviral activity of oxidized spermine with that of glutaraldehyde. It can be seen in Fig. 4 that glutaraldehyde, 0.62 mM, reduced the infective titer by 1.5 log units after incubation at 37 C for 5 hr, whereas oxidized spermine, at a concentration of 0.41 mM, caused a reduction by 3.5 log units under similar experimental conditions. Moreover, 1.25 mM glutaraldehyde behaved like 0.82 mM oxidized spermine; they both caused a reduction in infectivity of approximately 6 log units.

It has been claimed that the cytotoxicity and antiviral activity of oxidized spermine could be explained in terms of acrolein produced by its spontaneous degradation (1, 2, 13). To test this possibility, we studied the effect of this unsaturated aldehyde on the infectivity of vaccinia virus. This experiment showed that 0.40 mM acrolein reduced the infective titer of vaccinia virus by approximately 2 or 3 log units, after the respective incubation for 3 and 5 hr. These values are similar to those obtained for oxidized spermine (Fig. 2).

**DISCUSSION**

Previous studies in our laboratory showed that oxidized spermine inactivates bacterial (6, 7, 9), plant (8), and animal viruses (3-5, 12). These findings were confirmed in other laboratories (10, 14-16, 20). It has been demonstrated that oxidized spermine is unstable at alkaline pH or at high temperatures and that acrolein is one of its degradation products. Alarcon (1, 2) and Kimes and Morris (13) suggested that the acrolein formed by β-elimination reaction may explain the inhibitory action of oxidized spermine. If the biological activity of oxidized spermine resides in acrolein, then the activity of the latter should be at least twice that of oxidized spermine, since acrolein is formed at a 20 to 30% yield only. Results of the experiments described in this paper and elsewhere (5) do not support the hypothesis that acrolein is responsible for the antiviral activity of oxidized spermine. This also follows from other studies by Kremzner and Harter (14), who found that oxidized spermine was more toxic for vesicular stomatitis virus than was acrolein. Moreover, oxidized spermine is definitely more active than Formalin and glutaraldehyde in inactivating vaccinia virus.

It thus appears that the antiviral activity of oxidized polyamines is rather unique and cannot be simply explained by the presence of carbonyl groups in the active molecule. Previous studies (7) have shown that oxidized spermine penetrates into the viral particle and forms a stable complex with its deoxyribonucleic acid. This binding is apparently due to the cationic nature of the molecule, which has a high affinity for the anionic nucleic acid. In this respect, oxidized polyamines differ from mono- or dialdehydes which are not protonated and would therefore tend to react with surface proteins and not with the internal nucleic material.

**ACKNOWLEDGMENTS**

This study was supported by the S. Lunenfeld and R. Kunin Medical Research Foundation, and by a grant from the Wellcome Trust.

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**Fig. 4. Effect of glutaraldehyde on the infectivity of vaccinia virus.**
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