Inactivation of Reovirus Type 2 by a Combination of Chloroform and Moderate Temperature

RETO ENGLER AND CLARISA BROOME

Division of Microbiology, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D.C. 20204

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The infectivity and hemagglutinin activity (HA) of reoviruses are resistant to the action of diethyl ether (6, 8, 9). This resistance indicates that the coats of these viruses do not contain lipids or lipoproteins. Feldman and Wang (4) reported a good correlation between ether and chloroform sensitivities of a number of viruses and suggested that the two sensitivity tests could be interchanged for preliminary classification of viruses. Rozee and Leers (7), on the other hand, reported that chloroform inactivated HA but did not affect the infectivity of reovirus types 1, 2, and 3 within 30 min at room temperature (approximately 22 C). These latter findings, however, were not always reproducible, and it appeared that the temperature at which the chloroform treatment was performed played an important role. We, therefore, selected two defined temperatures, 0 C and 37 C, for the chloroform treatment.

Reovirus type 2 (D-5 Jones) was grown in monkey kidney tissue cultures which were maintained at 37 C in high-cysteine altered Eagle’s medium (1) without serum. The infected tissue cultures were harvested after 4 to 5 days by 3 cycles of freezing and thawing, and the cellular debris was removed by centrifugation at 1,600 × g for 25 min. This material was maintained at 4 C as stock suspension. For some experiments, the virus was purified by sedimentation in a Spinco model L2 65B ultracentrifuge (2 hr at 65,500 × g, no. 30 rotor), followed by banding of the resuspended pellet in a CsCl gradient (18 hr at 63,500 × g, SW-25 rotor).

Equal volumes (1 ml) of reovirus and chloroform were mixed in test tubes and incubated either in an ice-water bath at 0 C or in a water bath at 37 C. The water and chloroform phases were remixed every 3 to 5 min. The tubes were removed from the water baths at intervals ranging from 5 to 60 min and centrifuged for 1 min at 1,400 × g to separate the phases. The aqueous phase was withdrawn and transferred to tubes which were then stored at 0 C. At the time of transfer, the virus suspension was agitated by pipetting to permit evaporation of residual chloroform. Reovirus HA and infectivity titers (plaque-forming units/ml) were determined as previously described (2, 3).

The action of chloroform on reovirus type 2 was studied in at least 10 separate experiments. The following four controls were included each time: (i) reovirus kept at 37 C for 60 min, (ii) reovirus kept at 0 C for 60 min, (iii) reovirus kept at 37 C for 60 min in the presence of diethyl ether, and (iv) reovirus kept at 0 C for 60 min in the presence of diethyl ether. None of the controls showed any change in infectivity or HA when compared with the stock virus suspension. The results of a typical experiment are shown in Fig. 1. At 0 C, both infectivity and HA were resistant to the action of chloroform. Some inactivation of HA seemed to occur occasionally; however, the loss was never more than fourfold which may be within the limits of experimental error. At 37 C, the number of virus particles capable of forming plaques was reduced 1,000-fold and 10,000-fold after 30 and 60 min, respectively. The HA titer of 64 units was reduced to 2 units after 5 min. The HA seemed somewhat more chloroform-labile at 37 C since HA was almost completely inactivated after 5 min, whereas no appreciable loss of infectivity occurred during this same time interval. The chloroform sensitivities of purified virus and of virus from which only the cell debris had been removed were identical.

The results showed that reovirus type 2 was inactivated when treated with chloroform at 37 C. The inactivation was obviously due to the combination of chloroform and the moderate tem-
other viruses (4). Our results partially confirmed the findings by Rozee and Leers (7) in that HA was usually more quickly inactivated than infectivity by chloroform. On the other hand, complete inactivation of HA by chloroform without loss of infectivity could not be confirmed at the temperatures tested. The mechanism of the action of chloroform on the reovirus cannot be determined on the basis of these results; however, some form of denaturation rather than solubilization of the protein coat seems indicated.

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LITERATURE CITED