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Enhancement of the Infectivity of Rhinovirus Ribonucleic Acid by Diethylaminoethyl Dextran

MILAN FIALA AND BRUCE SALTZMAN

Department of Research Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

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Rhinoviruses are classified among the ribonucleic acid (RNA) viruses on the basis of the lack of inhibition of their infectivity by 5-fluorodeoxyuridine. Infectious RNA has been extracted with phenol from rhinovirus type 7 (5) and type 2 (3) and has been assayed on monolayers of diploid cells. In this study, infectious rhinovirus RNA was assayed on M-HeLa cells by a plaque test described previously (4). In this assay, diethylaminoethyl (DEAE) dextran was found to have a strong enhancing effect on the infectivity of rhinovirus RNA.

TABLE 1. *Effect of the concentration of DEAE dextran in the diluent upon the infectivity of rhinovirus type 2 RNA*

DEAE dextran (μg/ml)	Infectious RNA	
	PFU per 1:10 dilution of RNA extract	Per cent of highest titer
0	0	0
200	25	50
400	20	40
800	24	48
1,200	50	100
1,600	41	82
2,000	3	6
3,000	5	10

For isolation of infectious RNA, M-HeLa monolayers were infected with rhinovirus type 2 in a multiplicity of 5 to 10 plaque-forming units (PFU) per cell and were incubated at 35 C. Infected cells were harvested after 12 hr, and virus was released by 0.2% sodium dodecyl sulfate into LTM buffer (8) consisting of 0.14 M LiCl, 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (*p*H 7.4), and 0.001 M $MgCl_2$. This preparation of virus in LTM buffer was then extracted once with water-saturated phenol for 5 min at 60 C. RNA was precipitated from the aqueous phase by the addition at −20 C of three volumes of ethyl alcohol saturated with sodium chloride and several drops of a saturated solution of yeast RNA. The RNA precipitated was then centrifuged and washed once with ethyl alcohol. RNA extracted from 10^7 to 3×10^7 infected cells was redissolved in 5 ml of LTM buffer.

Initially, the hypertonic saline method (1) was tried unsuccessfully to assay the biological activity of the rhinovirus RNA. Treatment with 1 M NaCl was very toxic to M-HeLa cells after only 20 sec of contact. The infectious assay was made reproducible by the addition of DEAE dextran (Pharmacia, Uppsala, Sweden), of an approximate molecular weight of 2×10^6, to the LTM buffer used as a diluent of RNA. The concentration for optimal enhancement was between 1,200 to 1,600 μg/ml (Table 1). At concentration of 2,000 μg/ml or greater, DEAE dextran appeared toxic to cells at the end of the plaque assay. No plaques were produced by at least 1,000 PFU of RNA when DEAE dextran was omitted from the diluent. The plaque assay of infectious RNA was otherwise identical to that of the whole virus with M-HeLa monolayers and an overlay with 30 mM $MgCl_2$ (4). RNA was permitted to adsorb to cells at 35 C for 30 min. Plaques were revealed on the 3rd day. Plaque sizes were similar to those of plaques produced by whole virus.

TABLE 2. *Influence of the temperature during extraction by phenol upon the yield of infectious RNA*

Temp of extraction	Titer of RNA (PFU/ml) with different numbers of phenol extractions		
	1	2	3
C			
4	$10^{3.78}$	$10^{4.00}$	$10^{4.98}$
20	$10^{3.76}$	$10^{4.56}$	$10^{4.00}$
60	$10^{4.60}$	$10^{4.83}$	$10^{4.70}$

Several factors which are known to influence the yield of infectious RNA during extraction by phenol were evaluated: bentonite, DEAE dextran, and temperature. The presence of bentonite or of DEAE dextran during the extraction did not increase the titer of the RNA isolated. The temperature of extraction was important as the maximal yield was obtained following one extraction at 60 C, whereas three extractions were necessary at 4 C (Table 2). With one extraction at 60 C, the ratio of the titer of infectious RNA to that of starting virus was 10^{-4} to 10^{-6}.

TABLE 3. *Properties of the infectious rhinovirus RNA compared to the properties of whole rhinovirus*

Infectious prepn[a]	Treatment	Avg no. of remaining PFU
Rhinovirus type 2 RNA	None	22
	Ribonuclease, 0.25 μg/ml[b]	25
	Ribonuclease, 2.5 μg/ml	8
	Ribonuclease, 25.0 μg/ml	0
	Ribonuclease, 0.25 μg/ml[c]	10
	Ribonuclease, 2.5 μg/ml[c]	0
	Ribonuclease, 25.0 μg/ml[c]	0
	Deoxyribonuclease I, 25.0 μg/ml[b]	25
	Rhinovirus type 2 antiserum, 1:50	26
	Eagle's MEM, *p*H 3.3[d]	14
	Eagle's MEM, *p*H 7.0	24
Rhinovirus type 2	None	100
	Rhinovirus type 2 antiserum, 1:50	7
	Ribonuclease, 25.0 μg/ml	100
	Eagle's MEM, *p*H 3.3	0
	Eagle's MEM, *p*H 7.0	100

[a] RNA of rhinovirus type 2 or whole rhinovirus type 2, both diluted in LTM buffer containing DEAE dextran, were treated by twofold dilution in various reagents for 30 min at room temperature.

[b] From bovine pancreas, Worthington Biochemical Corp., Freehold, N.J.

[c] Diluted in LTM plus DEAE dextran, 300 μg/ml; all other preparations were diluted in LTM plus DEAE dextran, 800 μg/ml.

[d] Minimal essential medium.

To demonstrate that phenol did extract genetically active RNA from rhinovirus type 2, this extract was characterized by treatment with enzymes and with type-specific antiserum and by alteration of *p*H (Table 3). Whereas ribonuclease destroyed the infectivity of the phenol extract, it did not affect that of whole virus. DEAE dextran at the concentration of 800 μg/ml was partially protective of the extract against inactivation by ribonuclease.

The enhancement of infectivity of rhinovirus RNA by DEAE dextran is similar to the effect of this polycation on the infectious RNA of polio virus type 1 (6), of foot-and-mouth disease virus (2), and of mengovirus (7). DEAE dextran was found to protect rhinovirus RNA partially against inactivation by ribonuclease. This effect of DEAE dextran is similar to that found with poliovirus type 1 (6).

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