RECONSTITUTION OF POLIOVIRUS

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SUMMARY

Poliovirus, dissociated by exposure to 9 M urea and 0.09 M mercaptoethanol into RNA and protein, can be reconstituted to infectious viral particles by stepwise dilution with cold isotonic phosphate buffered saline at pH 7.2.

INTRODUCTION

Maturation of viruses may occur within the cell by a process of self-assembly of their protein and nucleic acid components (1). Therefore, it should be possible to obtain infectious virions *in vitro* by combining viral components under the appropriate conditions. While reconstitution of animal viruses has not been previously reported, such experiments have been successfully performed with plant (2,3) and bacterial (4,5) viruses. The efficiency of reconstitution of plant viruses approached 50 %. When bacteriophages were reconstituted, the efficiency of conversion of RNA strands with viral proteins to infectious particles was 2×10^{-6} (6).

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RESULTS

Poliovirus, type 1, strain "Mahoney", grown in S3-HeLa-cells and released by treatment of the infected cells with Triton N 101 (final concentration 0.25 %) was purified by differential centrifugation in the presence of 0.25 % sodium dodecylsulfate (7), resuspended in a solution of 0.5 g/ml of CsCl, extracted twice with chloroform and further purified by two zonal centrifugations in CsCl. The virus band was dialyzed against 0.15 M NaCl. Virus preparations containing 1×10^{11} plaque forming units per ml (PFU/ml) were diluted 1 : 10 in 10 M urea and 0.1 M β-mercaptoethanol and kept at 25° C for 60 minutes. After this time, no infectious virus could be demonstrated, at a dilution of 10^{-3} of the original sample (Table I, method a); the detection limit for intact virus in the sample was 1×10^{3} PFU/ml. However, urea-treated virus preparations contained infectious RNA at the same level as phenol-extracted viral RNA.

By the method of agar cell suspension plaque assay (8, 9), the infectivity of viral RNA can be titered by exposure of cells to DEAE-dextran (160 μg/ml) and dimethylsulfoxide (DMSO: 10 %) (Table I, method b). In the presence of infectious virions no discrimination between ribonucleic acid and virus can be made by this procedure. The presence of infectious RNA in the urea-dissociated sample was established by its sensitivity to pancreatic ribonuclease and by sucrose gradient centrifugation. RNase added after the urea-mercaptoethanol treatment of the virus destroyed the infectivity of the RNA as measured by method b. After urea dissociation, ^{3}H-uridine-labeled virus particles in sucrose gradients revealed a compo-

TABLE I

Inactivation of poliovirus by urea

Treatment	Temperature	Infectivity PFU/ml	
		Virus Method a	RNA Method b
9 M urea	25°	$< 1 \times 10^3$	7×10^6
9 M urea + 0.09 M mercaptoethanol	25°	$< 1 \times 10^3$	6×10^6
9 M urea	37°	$< 1 \times 10^3$	n.t.+
9 M urea + 0.09 M mercaptoethanol	37°	$< 1 \times 10^3$	2.7×10^6
Phosphate buffered saline	37°	1×10^{11}	2×10^{11}

Twenty μl of purified and concentrated poliovirus (1×10^{11} PFU/ml) in 0.15 M NaCl solution were mixed with 200 μl of 10 M urea with or without 0.1 M β-mercaptoethanol in H_2O and kept at 25°C or 37°C in stoppered glass tubes for 60 minutes. The samples were diluted at least 10-fold with cold isotonic phosphate buffered saline (0.02 M sodium potassium phosphate buffer pH 7.2 with 0.12 M NaCl) and 100 μl samples tested for infective virus particles (a) or 200 μl samples for infectious viral RNA (b), as described (8,9). The control was kept in isotonic phosphate buffered saline.

+ n.t. = not tested

nent sedimenting exactly in the same fraction as phenol-extracted poliovirus RNA.

Attempts to reconstitute infectious virus by dialysis of dissociated virus samples against solutions of isotonic phosphate buffered saline, pH 7.2, were unsuccessful. However, a 1000- to 5000-fold increase in infectivity over the detection limit was obtained by a stepwise dilution of urea-treated virus preparations with cold phosphate buffered saline (Table II, method a).

TABLE II

Reactivation of urea-dissociated poliovirus

Treatment	Infectivity PFU/ml	
	Virus Method a	RNA Method b
9 M urea + 0.09 M mercaptoethanol, 60 min. 25°	$<1 \times 10^4$	6×10^6
Dilution 1 : 2 with phosphate buffered sal.	$<2 \times 10^4$	5×10^6
Dilution 1 : 4 "	$<4 \times 10^4$	5×10^6
Dilution 1 : 8 "	6×10^7	5×10^7
Dilution 1 : 16 "	5×10^7	5×10^7
Dilution 1 : 32 "	4×10^7	8×10^7

Poliovirus was inactivated by treatment with urea-mercaptoethanol (as described in Table I), tested for infectivity and diluted stepwise at 4°C with cold isotonic phosphate buffered saline, pH 7.2. The infectivity of the virus (a) and viral RNA (b) was measured (see Table I).

No infective particles were detected if the virus sample was treated with pancreatic RNase prior to the dilution procedure. In contrast, if the RNase treatment was performed after virus reconstitution had occurred, no decrease in infectivity was observed (Table III). Rate zonal centrifugation of native and reconstituted poliovirus in sucrose gradients revealed that the infectivity of both samples was found exactly at the 160S position in the gradient (10). These experiments imply that a reconstitution into complete virus particles has occurred. The infectivity of the reconstituted virus compared to the infectivity of the virus used for the dissociation procedure is in the range of max. 0.05 % (Tables I and II). Since

TABLE III

Effect of ribonuclease on inactivated and reactivated poliovirus

No.	Treatment of the sample	RNase added before/after reactivation	Infectivity PFU/ml Virus Method a	RNA Method b
1	9 M urea + 0.09 M mercaptoethanol	-	$< 1 \times 10^4$	2×10^7
2	9 M urea + 0.09 M mercaptoethanol	+	$< 1 \times 10^4$	$< 1 \times 10^4$
3	Diluted 1 : 8	-	3×10^7	6×10^7
4	Diluted 1 : 8	+	$< 8 \times 10^4$	$< 8 \times 10^4$
5	Reactivated virus	-	2×10^7	n.t.+
6	Reactivated virus	+	3×10^7	n.t.+

Poliovirus was inactivated and reactivated by a 1 : 8 dilution with cold isotonic phosphate buffered saline (as described in Tables I and II). In samples Nos. 2 and 4 pancreatic ribonuclease (20 μg/sample) was added prior to the dilution step. Reactivated virus (No. 6) was treated with 50 μg of pancreatic ribonuclease dissolved in water. Infectivity measurements were done and expressed as described in Table I.

+ n.t. = not tested

the efficiency of detection of RNA infectivity is also 0.05 % of that of viruses (9), all of the infectious RNA is converted into infectious virus.

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