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Influence of Salts on Electrostatic Interactions Between Poliovirus and Membrane Filters

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Neither solutions of salts nor solutions of detergents or of an alcohol at pH 4 are capable of eluting poliovirus adsorbed to membrane filters. However, solutions containing both a salt, such as magnesium chloride or sodium chloride, and a detergent or alcohol at pH 4 were capable of eluting adsorbed virus. The ability of ions to promote elution of virus at low pH in the presence of detergent or alcohol was dependent on the size of the ions and the ionic strength of the medium. These results suggest that both electrostatic and hydrophobic interactions are important in maintaining virus adsorption to membrane filters. Hydrophobic interactions can be disrupted by detergents or alcohols. It appears that electrostatic interactions can be disrupted by raising the pH of a solution or by adding certain salts. Disruption of either electrostatic or hydrophobic interactions alone does not permit efficient elution of the adsorbed virus at low pHs. However, when both interactions are disrupted, most of the poliovirus adsorbed to membrane filters is eluted, even at pH 4.

The parameters which influence the association of viruses with membrane filters have been determined in several studies (1, 13, 17). The pH of a solution has been found to be a major factor influencing viral adsorption to membrane filters (13, 15, 18). At pH values below the isoelectric point of the virus, viruses often have a net positive charge, whereas certain membrane filters have a net negative charge (10, 11, 15). Under these conditions, adsorption may be due to electrostatic interactions between the viruses and the filter. At pH values above the isoelectric point of the virus, both the virus and the membrane filters possess a net negative charge (9, 11, 15). Adsorption of viruses to membrane filters under these conditions is usually minimal. However, the presence of certain salts permits adsorption at these high pH values that normally would prevent viral adsorption (6, 16, 17). This phenomenon has been discussed elsewhere (13, 18), and it has been suggested that salts alter electrostatic interactions between viruses and filters.

It has been shown recently that certain salts promote the association of viruses with membrane filters by strengthening hydrophobic rather than electrostatic interactions (4). Detergents, such as Tween 80, which are capable of disrupting hydrophobic interactions, promote elution of viruses adsorbed to membrane filters (6, 18). Therefore, this study was undertaken to determine whether certain salts which have been shown to promote hydrophobic interactions (6–8) could overcome the effects of detergents and promote viral adsorption in the presence of Tween 80. Contrary to the expected results, it was found that the addition of salts to Tween 80 solutions at low pH actually promoted elution of viruses adsorbed to membrane filters.

MATERIALS AND METHODS

Virus and viral assays. Poliovirus type 1 (strain LSc) was used in all tests. Virus was assayed on BGM cells by a routine plaque procedure, using 1.5% methylcellulose in Eagle minimal essential medium supplemented with 5% fetal calf serum as an overlay. Plaques were visualized with a 1:10,000 dilution of neutral red after a 48-h incubation. Virus was diluted in Eagle minimal essential medium with 2% fetal calf serum before the assay.

Membrane filters. Nitrocellulose membrane filters (Millipore type HA; Millipore Corp., Bedford, Mass.) were used in adsorption-elution studies. All filters were contained in 25-mm holders and rinsed before being tested with 10 ml of deionized water. In addition, filters used in experiments with *tert*-butyl alcohol were rinsed with a 30% solution of the alcohol.

Chemicals. The chemicals used in this study and their sources were as follows: potassium hydrogen phthalate (KHP), sodium chloride, calcium chloride, magnesium chloride, monobasic potassium phosphate, and dibasic potassium phosphate from Fisher Scientific Co., Fair Lawn, N.J.; trichloroacetic acid, monochloroacetic acid, cetyltrimethylammonium bromide, Triton X-100, and Tween 80 from Sigma Chemical Co., St. Louis, Mo.; *tert*-butyl alcohol, sodium acetate, and sodium citrate from Mallinckrodt, Inc., Paris, Ky.; and beef extract from Inolex Corp., Glenwood, Ill. Solutions were adjusted to the desired pH

TABLE 1. Elution of poliovirus type 1 adsorbed to membrane filters

Solution[a]	% of adsorbed virus eluted
Buffer alone	0
0.05% Tween 80	21
0.05% Tween 80 + 0.5 M sodium citrate	77
0.05% Tween 80 + 1.0 M $CaCl_2$	95
0.1% cetyltrimethylammonium bromide	92

[a] All solutions contained 0.05 M KHP buffer and were adjusted to pH 4.

by the addition of hydrochloric acid or sodium hydroxide.

Adsorption and elution studies. A solution of 0.05 M monobasic potassium phosphate was adjusted to pH 4 with HCl. After ca. 10^5 PFU of virus was added, 10 ml of the solution was passed through the filters at a rate of approximately 1 ml/s. Viruses in the initial solution and in the filter effluent were assayed to confirm retention by the filter. Next, 10 ml of a test eluent (see tables) was passed through the filters, and the eluted viruses were assayed. Residual virus was eluted with 10 ml of 3% beef extract (pH 9) and assayed. The virus eluted was expressed as a percentage of the virus present in the initial test solution. Values were obtained in two trials and represents the means of four determinations.

RESULTS

Poliovirus 1 was adsorbed to membrane filters in the presence of monobasic potassium phosphate, the pH of which was adjusted to 4. Viral adsorption was virtually 100%, with little (<1%) or no virus detected in the filter effluent. The results of preliminary studies on the effects of salts on the ability of Tween 80 solutions to elute virus adsorbed to membrane filters are presented in Table 1. KHP at pH 4 did not elute detectable levels of virus. A solution containing a neutral detergent (Tween 80) eluted less than 25% of the adsorbed poliovirus. However, addition of a divalent salt ($CaCl_2$) or a metal chelator (sodium citrate) to the Tween 80 solution increased the amount of virus eluted to greater than 75%. Cetyltrimethylammonium bromide, a cationic detergent, alone was effective in eluting 92% of the virus. Because these preliminary results demonstrated that salts enhance the ability of Tween 80 solutions to elute virus, the effect of detergent or alcohol concentration on viral elution was then determined.

A threshold of viral elution was observed when the concentration of Tween 80 was increased in the NaCl solutions. Tween 80 concentrations of 0.005% and above brought about at least a 90% elution efficiency, whereas solutions with 0.001% Tween 80 or with no detergent eluted less than 10% of the virus adsorbed to membrane filters. A 3% beef extract solution adjusted to pH 9 was used as a control measure to recover any virus not removed by treatment with the primary eluent. The recovery of virus by this step showed that the virus was not inactivated or aggregated by the primary eluent (Table 2).

Another detergent or *tert*-butyl alcohol could be substituted for Tween 80. The results for these solutions were similar to those obtained for Tween 80. Solutions containing only 0.1% Triton X-100 or 2.0 M *tert*-butyl alcohol eluted little virus. However addition of 1.0 M NaCl to these solutions permitted elution of essentially all virus adsorbed to the filters (Table 3).

The ability of solutions containing Tween 80 and salts buffered at pH 4 to elute adsorbed virus was related to the ionic strength of the solution (Fig. 1). Solutions with 0.1% Tween 80 and ionic strengths of greater than 0.3 were able to elute greater than 90% of the adsorbed virus. Decreasing the ionic strength reduced the amount of virus eluted. Treatment of these filters with solutions of 3% beef extract (pH 9) caused elution of the remaining virus (data not shown), again indicating that the virus was not being inactivated by the low-ionic-strength solutions.

The influence of anions in solutions of 0.1% Tween 80 buffered at pH 4 on the elution of

TABLE 2. Influence of Tween 80 concentration on elution of poliovirus type 1 adsorbed to membrane filters in the presence of 1.0 M NaCl at pH 4

% Tween 80	% of adsorbed virus eluted by:			
	Primary eluent		3% beef extract, pH 9	
	Mean	SD	Mean	SD
0[a]	0	0	87	20
0.001	8	7	101	5
0.005	91	7	13	4
0.01	93	7	7	5
0.05	99	4	4	2
0.1	104	7	3	2

[a] All solutions contained 1.0 M NaCl buffered with 0.05 M KHP adjusted to pH 4.

TABLE 3. Effects of detergents and *tert*-butyl alcohol on the elution of poliovirus type 1 adsorbed to membrane filters

Primary eluting solution[a]	% of adsorbed virus eluted by:			
	Primary eluent		3% beef extract, pH 9	
	Mean	SD	Mean	SD
Buffer alone[b]	0	0	107	7
0.1% Tween 80[b]	13	6	91	11
0.1% Tween 80 + 1.0 M NaCl[b]	106	4	9	7
2.0 M *tert*-butyl alcohol	0	0	114	20
2.0 M *tert*-butyl alcohol + 1.0 M NaCl	103	13	14	2
0.1% Triton X-100	17	3	88	9
0.1% Triton X-100 + 1.0 M NaCl	140	16	2	1

[a] All solutions contained 0.05 M KHP buffer and were adjusted to pH 4.
[b] Values from Table 5.

poliovirus adsorbed to membrane filters is shown in Table 4. Anions of low-molecular-weight sodium salts were more effective in bringing about viral elution when added to solutions of Tween 80. Chloride (molecular weight, 35.4) eluted 77% of the adsorbed virus, and acetate (molecular weight, 59.1) eluted 80% of the adsorbed virus. In contrast, anions of higher molecular weight were not as efficient in bringing about viral elution in the presence of detergent. Monochloroacetate (molecular weight, 93.5) eluted only 22% of the adsorbed virus, and trichloroacetate (molecular weight, 162.4) eluted only 27% of the adsorbed virus. Solutions of the buffer, 0.1% Tween 80, or the anions alone eluted less than 15% of the adsorbed poliovirus (data not shown).

Both the pH and the nature of the solution played a major role in the elution of the viruses from membrane filters (Table 5). At pH 4, solutions of buffer, 0.1% Tween 80, or 1.0 M NaCl eluted less than 13% of the adsorbed virus. However, a solution of 0.1% Tween 80 and 1.0 M NaCl eluted 100% of the adsorbed virus even at this low pH. At pH 7, the buffer eluted 35% of the adsorbed virus and 1.0 M NaCl eluted only 12%. At this higher pH, the solution of 0.1% Tween 80 and the solution containing both the detergent and salt each eluted over 85% of the adsorbed virus. Even at pH 9, solutions of 1.0 M NaCl continued to elute only a small percentage (36%) of adsorbed virus. The other solutions tested eluted more than 65% of the adsorbed virus at this pH.

DISCUSSION

Several factors influence the association of viruses with membrane filters. These factors include pH (13, 15, 18), the concentration and type of salt in solution (17, 18), the presence of organic compounds such as proteins and humic compounds (18), the composition and porosity

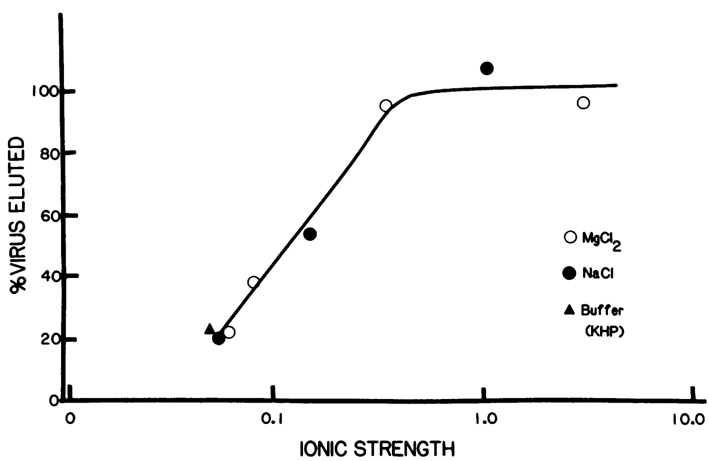

FIG. 1. Elution of poliovirus type 1 adsorbed to membrane filters by solutions of Tween 80 and NaCl or $MgCl_2$ at pH 4.

TABLE 4. Influences of anions on elution of poliovirus type 1 to membrane filters

Anion[a]	Mol wt	% of Adsorbed virus eluted by:			
		Primary eluent		3% beef extract, pH 9	
		Mean	SD	Mean	SD
Chloride	35.4	77	14	16	13
Acetate	59.1	80	12	17	13
Monochloroacetate	93.5	22	2	62	15
Trichloroacetate	162.5	27	3	74	3

[a] All anions were used as sodium salts at 0.5 M in the presence of 0.1% Tween 80 and 0.05 M KHP, adjusted to pH 4.

of the filter (15), and the flow rate of fluid through the filter (14). Accordingly, these factors have been studied both to better understand virus-membrane filter interactions and to develop better methods for recovering viruses from water.

The results of previous studies have shown that at pH values below the virus isoelectric point, poliovirus is positively charged, whereas membrane filters such as the Millipore filters used in this study are negatively charged. The resulting electrostatic attraction is likely to be a factor promoting viral adsorption (10, 11, 15). At pH values above the virus isoelectric point, the virus and the filters have net negative charges and viral adsorption is minimal. However, the addition of certain cations has been found to increase viral adsorption at these higher pH values, where repulsive forces between the negative charges on the viruses and the filter may dominate (12, 16, 17). Three possible mechanisms for the observed enhancement of virus adsorption have been suggested. It has been proposed that the positively charged cations act as bridges between the negative charges on the viruses and the filters (10). A second suggestion is that the cations are adsorbed by the filters and reverse the net charge of the filter from negative to positive (12). Another possibility is that the addition of cations (and therefore an increase in ionic strength) reduces the diffuse layer of ions surrounding the viruses and the filter. This lets short-range attractive forces overcome the electrostatic barriers and permits adsorption of the viruses (16). These explanations suggest that electrostatic interactions are altered by the addition of salts.

In addition to their effects on electrostatic interactions, salts have been shown to influence hydrophobic interactions. Hatefi and Hanstein (7) were able to increase the solubility of certain proteins by using solutions of chaotropic ions. These chaotropic ions are relatively large, singly charged ions such as trichloroacetate, thiocyanate, and iodide (8). It has been suggested that these ions decrease the structure of water and therefore make aqueous solutions more lipophilic. Solutions of chaotropic ions have been found to solubilize membrane proteins and organic compounds such as riboflavin and adenine and

TABLE 5. Effects of pH, NaCl, and Tween 80 on elution of poliovirus type 1 adsorbed to membrane filters

pH	Primary eluting solution	% of adsorbed virus eluted by:			
		Primary eluent		3% beef extract, pH 9	
		Mean	SD	Mean	SD
4	Buffer alone[a]	0	0	107	7
	0.1% Tween 80	13	6	91	11
	1.0 M NaCl	2	0	111	3
	0.1% Tween 80 + 1.0 M NaCl	106	4	9	7
7	Buffer alone	35	8	63	28
	0.1% Tween 80	86	11	4	4
	1.0 M NaCl	12	4	91	2
	0.1% Tween 80 + 1.0 M NaCl	92	3	4	3
9	Buffer alone	66	10	37	11
	0.1% Tween 80	66	8	2	1
	1.0 M NaCl	36	3	92	2
	0.1% Tween 80 + 1.0 M NaCl	92	9	3	3

[a] All solutions contained 0.05 M KH_2PO_4 as a buffer and were adjusted to the proper pH value.

to disrupt antigen-antibody complexes (3, 4, 7).

In contrast, antichaotropic ions are generally small, singly charged ions such as fluoride or multivalent ions such as citrate, calcium, or magnesium. These antichaotropic ions have been found to promote hydrophobic interactions, presumably by increasing water structure (7, 8). By increasing water structure, the ability of solutions to accommodate hydrophobic groups is reduced, and hydrophobic interactions between apolar groups in the solution are increased. These antichaotropic salts have been shown to counteract the ability of chaotropic salts to disrupt hydrophobic interactions (4, 6–8).

Recent studies have shown that chaotropic salts promote elution of viruses adsorbed to membrane filters (6; R. S. Moore, D. A. Wait, and E. H. Stokes, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, Q55, p. 219) and estuarine sediments (D. A. Wait, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, Q53, p. 218). Un-ionized compounds such as Tween 80 and urea have also been used to elute viruses adsorbed to membrane filters, estuarine and freshwater sediments, and wastewater sludges (2, 5). Solutions containing antichaotropic ions such as citrate, EDTA, and magnesium have been found either to promote virus adsorption to membrane filters or to be relatively ineffectual in eluting the viruses adsorbed to the filters (4, 17).

The results obtained in studies with proteins and viruses suggest that chaotropic salts and unionized compounds such as Tween 80, urea, and ethanol disrupt hydrophobic interactions, whereas antichaotropic salts promote such interactions (3, 4, 6–8). The purpose of this study was to determine whether the ability of certain salts to promote hydrophobic interactions could overcome the disruption of hydrophobic interactions caused by Tween 80. It was hoped that by adding certain salts to solutions of Tween 80, viral adsorption would occur even in the presence of the detergent. However, the addition of the antichaotropic salts citrate and $CaCl_2$ did not promote viral adsorption as expected, but in fact, promoted efficient viral elution. In addition, cetyltrimethylammonium bromide, a positively charged detergent, was much more efficient in eluting adsorbed virus than its nonionic counterpart. These results can be explained by assuming that electrostatic and hydrophobic interactions are crucial in maintaining virus-filter association at low pH. When a neutral detergent disrupts hydrophobic interactions, then addition of a charged species is able to disrupt electrostatic interactions and bring about elution of the virus. Alternatively, the charge can be incorporated as a part of the detergent, and elution is also a result.

Both the ionic strength and the type of ions present influenced elution of adsorbed virus at low pH in the presence of Tween 80. Solutions of $MgCl_2$ or NaCl with ionic strengths of greater than 0.3 eluted most of the adsorbed virus.

The anions of sodium salts differed in their abilities to elute virus at pH 4 in the presence of Tween 80. The anions of low molecular weight, such as chloride and acetate, were more effective in eluting virus adsorbed to filters than were anions of higher molecular weight. These results are consistent with the effect of ions on electrostatic interactions as discussed by Kauzmann (9). The effect of an ion on electrostatic interactions is proportional to the charge on the ion and inversely proportional to the ionic radius of the ion. In other words, the effect of an ion on electrostatic interactions is related to the charge density of the ion. Therefore, ions with smaller ionic radii (higher charge density) would be likely to have a greater effect on electrostatic interactions than larger ions with the same net charge.

At different pH values, the contributions of electrostatic and hydrophobic interactions vary. At high pH values (pH 9 through 11), both the virus and the nitrocellulose filters are negatively charged (10, 11, 15). Solutions having minimal effects on hydrophobic interactions (buffer) or solutions capable of disrupting hydrophobic interactions (Tween 80) promote efficient viral elution. A solution of NaCl, which by itself can strengthen hydrophobic interactions, eluted less virus than the buffer solution alone. Our results agree with a previous study, which showed that at high pH, hydrophobic interactions may be the major factor in maintaining virus-filter adsorption (6).

At low pH (pH 4), solutions which disrupt only electrostatic interactions or solutions which disrupt hydrophobic interactions alone did not elute appreciable amounts of virus. At this low pH value, only solutions containing both types of compounds eluted virus efficiently. Since viral adsorption is generally facilitated at low pH, it is possible to adsorb and elute virus with no change in pH by varying the concentrations of detergent and salt.

Several previous studies have shown that salts promote adsorption to membrane filters (1, 13, 11). Results of a recent study suggest that such salts promote hydrophobic interactions between viruses and filters (4). This explanation is consistent with our results. The results of our study suggest that salts can disrupt electrostatic interactions between virus and membrane filters if hydrophobic interactions are also disrupted. If hydrophobic interactions are not disrupted, solutions of certain salts such as $MgCl_2$ or NaCl alone will promote viral adsorption. This is likely due to their effect on strengthening hydrophobic interactions. If hydrophobic interactions are disrupted, then the disruption of electrostatic interactions by the salts results in viral elution.

Preliminary results from our laboratory have shown that viruses vary in the amount of hydrophobic and electrostatic interactions with membrane filters. This may permit development of simple procedures for separating virus types. Further studies should be done to determine the relative contributions of electrostatic and hydrophobic interactions to virus-filter associations.

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