Proc. Natl. Acad. Sci. USA
Vol. 78, No. 2, pp. 1229–1232, February 1981
Microbiology

Effects of chaotropic and antichaotropic agents on elution of poliovirus adsorbed on membrane filters

(virus/surfactant/hydrophobic interactions/electrostatic interactions)

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Communicated by George K. Davis, October 20, 1980

ABSTRACT **The association of poliovirus with membrane filters results from both electrostatic and hydrophobic interactions. At low pH, electrostatic interactions appear to dominate. However, at high pH, hydrophobic interactions appear to dominate with both Millipore and Zeta plus filters. With both filters, viral elution was prevented at high pH by the presence of antichaotropic salts, which strengthen hydrophobic associations. This effect was antagonized by detergents and by chaotropic salts, which weaken hydrophobic associations. Excellent correlation was observed between viral elution, the solubilization of adenine, and the micelle-formation process in the presence of chaotropic and antichaotropic agents. Such simple measurements of the relative ability of solvents to accomodate hydrophobic groups may be of predictive value in designing methods for concentration and purification of viruses. The hypothesis that hydrophobic interactions are the primary force involved in the stabilization of virus attachment at high pH is consistent with observations that cannot be explained by consideration of electrostatic interactions as the major stabilizing factor.**

Conditions that promote viral adsorption on and elution from solids have been determined empirically in studies performed in different laboratories over the last 20 years.

Electrostatic interactions between charged groups on the solids and the viruses have been used to explain these results. Low pH has been found to promote the association of viruses with solids such as membrane filters (1), activated carbon (2), and soils (3). Viruses have a net positive charge at pH values less than their isoelectric point (7 for poliovirus, ref. 4). Because most solids and membrane filters have a net negative charge at pH values <3 (3, 5), virus retention at low pH can usually be explained as due to an attraction between oppositely charged groups. High pH has been generally found to interfere with virus–solid interactions, and solutions buffered at pH 9–11.5 have been used to elute viruses from membrane filters (1). At high pH values, both the filter and the virus have net negative charges (4, 5), so attractive forces should be minimal. However, many solutions fail to elute viruses even at high pH (6, 7). This effect is thought to result from salt bridges between negatively charged groups on the viruses and the filter or from attractive forces between the viruses and cations adsorbed on the filters (1, 5). The addition of solutions containing di- and trivalent cations have been found to promote retention of viruses by solids (1, 3).

The failure of citrate ion (6) or EDTA (7, 8) to elute viruses from membrane filters at high pH is difficult to explain in terms of electrostatic interactions between the viruses and the filters only. The presence of chelating agents should reduce the ability of multivalent metal ions to act as bridges between the negatively charged groups on the virus and the filter or to alter the charge on the filter surface. Other experimental results are also difficult to explain solely on the basis of such electrostatic interactions. For example, solutions of beef extract or casein at pH 9 are capable of eluting viruses associated with Millipore and Zeta plus membrane filters. However, solutions of hydrolyzed protein (casamino acids) or of an individual amino acid (aspartic acid) at the same concentration and pH do not elute viruses from these filters (7). Further, the nonionic compound urea (7) and the detergents sodium lauryl sulfate and Tween 80 (1, 8) promote elution of virus.

In an attempt to explain the variable effects of different solutions on the elution of viruses from membrane filters, we have explored hydrophobic interactions as an alternative to the hypothesis of electrostatic interactions for viral retention at high pH. In these studies, we examined the effects of chaotropic and antichaotropic salts (9) on the elution of viruses adsorbed to membrane filters.

MATERIALS AND METHODS

Virus and Viral Assays. Poliovirus-1 (strain LSc) was used in all tests. Viruses were assayed by using MA-104 cells and a methylcellulose overlay as described (6).

Membrane Filters. Nitrocellulose membrane filters (Millipore type HA, Millipore) and filters composed of diatomaceous earth and ion-exchange resin (Zeta plus C-30, AMF, Meriden, CT) were used in adsorption-elution studies. All filters were contained in 25-mm holders.

Chemicals. The chemicals used in this study were as follows: glycine, sodium fluoride, sodium iodide, trichloroacetic acid, Tween 80, and Triton X-100 were from Sigma; sodium chloride, sodium sulfate, and sodium phosphate were from Fisher Scientific; beef extract was from Inolex, Glenwood, IL; magnesium 9-anilinonapthalene-1-sulfonate was from Kodak. All chemicals used in elution studies except beef extract were dissolved in 0.02 M glycine and adjusted to pH 9.5 by addition of sodium hydroxide or to pH 4 by addition of hydrochloric acid. Beef extract was used at 3% (g/100 ml) at pH 9.

Adsorption-Elution Studies. Tap water was dechlorinated by the addition of sodium thiosulfate (1 mg per liter) and adjusted with 0.1 M HCl to pH 3.5 for use with Millipore filters and to pH 6.5–7 for use with Zeta plus filters. After addition of approximately 10^4 plaque-forming units of poliovirus, 10 ml of the water was passed through the filters. Viruses in the initial sample and in the filter effluent were assayed to confirm retention by the filter. Then, 10 ml of a test eluent (see Tables 1 and 2) was passed through the filters, and the eluted virus was assayed. Any remaining viruses were eluted with 10 ml of 3% beef extract (pH 9) and assayed. Results are expressed as percentage of total virus initially retained by the filter.

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Abbreviation: CMC, critical micelle concentration.

Determination of Critical Micelle Concentration. Critical micelle concentrations (CMCs) were determined by the fluorescence method essentially as described by Horowitz (10). The neutral detergent Triton X-100 was used instead of sodium dodecyl sulfate to minimize the effects of charge neutralization. Titrations were done manually by adding microliter quantities of 0.4% Triton X-100 to 2.0 ml of naphthalene sulfonate-containing eluent. Fluorescence measurements were made by using an Aminco fluorocolorimeter. Samples were excited at 360 nm by using an Aminco J4-7113 excitation filter, and emission was measured above 495 nm by using a combination of two Aminco J4-7160 and one Aminco J4-7163 filters. Values were corrected for small concentration changes on addition of titrant.

Determination of Adenine Solubility. To determine the solubility of adenine, test solutions (20 ml) were incubated at 22°C overnight in a reciprocating shaker with 100 mg of adenine. Undissolved adenine was removed by centrifugation for 10 min at 6000 × *g*. The solution was then diluted (1:1000) into distilled water and dissolved adenine was determined by measuring the absorbance at 260 nm. These measurements were corrected for the minor changes in molar absorption introduced by the different salts and are expressed as percentage relative to buffer alone.

RESULTS

Effects of Chaotropic and Antichaotropic Agents on Viral Elution. At pH 4, adsorbed viruses were firmly attached to membrane filters and were not eluted by buffer alone (Table 1). At this pH, the elution of viruses was not facilitated by the addition of high concentrations (0.6 M) of NaCl, the chaotropic salt Cl_3CCO_2Na, or the antichaotropic salt NaH_2PO_4. At pH 9, however, buffer alone was sufficient for viral elution (Table 2). High concentrations (0.6 M) of the antichaotropic salts, NaF and Na_2HPO_4, retarded viral elution from the Millipore filters and prevented elution from the Zeta plus filters at high pH. Similar concentrations of the chaotropic salts Cl_3CCO_2Na and NaSCN did not retard elution from either type of filter. NaI was intermediate in its ability to prevent elution at high pH, consistent with the weakly chaotropic properties of I^- (9). None of the solutions examined inactivated or aggregated virus at either high or low pH. At low pH, viruses not eluted by the test solution were recovered in the second elution, with beef extract (see Table 1). Similar results were obtained at high pH (not shown).

Table 1. Elution of poliovirus adsorbed on membrane filters by buffered salt solutions at pH 4 and beef extract

Filter	Primary eluent	Virus eluted by primary eluent, %	Virus eluted by second eluent, %
Millipore	Glycine (0.02 M)	8 ± 6	80 ± 6
	Cl_3CCO_2Na	0	88 ± 3
	NaCl	2 ± 2	79 ± 12
	NaH_2PO_4	5 ± 1	84 ± 12
Zeta plus C-30	Glycine (0.02 M)	20 ± 5	89 ± 11
	Cl_3CCO_2Na	12 ± 2	90 ± 2
	NaCl	0	92 ± 7
	NaH_2PO_4	1 ± 21	89 ± 6

Values represent mean (±SD) of duplicate determinations.

We also examined the effects of mixtures of chaotropic and antichaotropic agents on viral elution at high pH by using Zeta plus filters. Viral elution was prevented by 0.2 M Na_2HPO_4 and by 0.2 M NaF in buffer and by mixtures of them. However, the addition of 0.4 M Cl_3CCO_2Na partially antagonized the effects

Table 2. Elution of poliovirus adsorbed on membrane filters by buffered salt solutions at pH 9.5 and beef extract

Filter	Trials, no.	Primary eluent	Virus eluted by primary eluent (mean ± SD), %	Adenine solubility,* %	CMC, mg/100 ml
Millipore	3	0.02 M Glycine buffer	85 ± 5	100	10.0
	6	0.6 M Cl_3CCO_2Na	96 ± 5	147	8.0
	3	0.6 M NaI	79 ± 15	103	7.3
	6	0.6 M NaF	42 ± 31	69	3.4
	2	0.6 M Na_2HPO_4	0	73	1.4
Zeta plus C-30	3	0.02 M Glycine buffer	93 ± 12	100	10.0
	3	0.6 M Cl_3CCO_2Na	92 ± 11	147	8.0
	2	0.6 M NaSCN	94 ± 3	106	8.8
	2	0.6 M NaI	64 ± 20	103	7.3
	3	0.6 M NaCl	1 ± 1	88	4.0
	3	0.6 M NaF	2 ± 2	69	3.4
	2	0.6 M Na_2HPO_4	2 ± 1	73	1.4
	2	0.2 M Na_2HPO_4	3 ± 1	93	4.2
	2	0.2 M NaF	7 ± 15	85	5.7
	2	0.2 M $NaHPO_4$ +0.4 M NaF	2 ± 1	82	1.8
	2	0.2 M Na_2HPO_4 +0.4 M Cl_3CCO_2Na	77 ± 6	117	4.4
	3	0.2 M NaF +0.4 M Cl_3CCO_2Na	56 ± 22	110	5.3
	2	0.2 M Na_2HPO_4 +0.1% Tween 80	65 ± 4	104	ND

ND, not done.
* Relative to that of buffer (0.950 g/liter).

of 0.2 M NaF and of 0.2 M Na_2HPO_4 and facilitated the partial elution of viruses. The detergent Tween 80 (0.1%) also partially antagonized the effects of 0.2 M Na_2HPO_4, facilitating partial elution.

Effects of Chaotropic and Antichaotropic Agents on Ability of Eluent Buffer To Accommodate Hydrophobic Groups in Solution at High pH. Although the general chaotropic activities of the salts used in this study have been determined (9), the specific effects of these salts on the glycine buffer (pH 9.5) have not been examined. The relative ability of the test eluents to accommodate hydrophobic groups in solution was determined by measuring the solubility of adenine (9) and by determining the CMC of Triton X-100 (see Table 2). Antichaotropic salts (0.6 M) such as NaCl, NaF, and Na_2HPO_4 caused a decrease in the solubility of adenine and a large decrease in the concentration at which Triton X-100 forms micelles relative to micelle formation in buffer alone. Chaotropic salts (0.6 M) such as Cl_3CCO_2Na and NaSCN caused an increase in the solubility of adenine and only a small decrease in the CMC of Triton X-100. Again, NaI appeared intermediate between the two groups. These results are in excellent agreement with previous studies (9) and suggest that antichaotropic salts decrease the ability of the buffer to accommodate hydrophobic groups and that chaotropic salts have little effect on micelle formation or increase (adenine solubility) the ability of buffer to accommodate hydrophobic groups.

The adenine solubility trends confirm the opposite effects of chaotropic and antichaotropic agents (9). However, both chaotropic and antichaotropic agents caused similar shifts in CMC (see Table 2) although the antichaotropic agents had much greater effects. We believe that the differences between the effects on adenine solubility and on the CMC are probably due to polar group interactions within the micelles, which are influenced by salts.

Lower concentrations (0.2 M) of NaF and Na_2HPO_4 also decreased the solubility of adenine, and a mixture of NaF and Na_2HPO_4 decreased it further (see Table 2). The decrease in adenine solubility caused by 0.2 M NaF and by 0.2 M Na_2HPO_4 was antagonized by 0.4 M Cl_3CCO_2Na and by detergent, analogous to the antagonistic effects of these agents in viral elution.

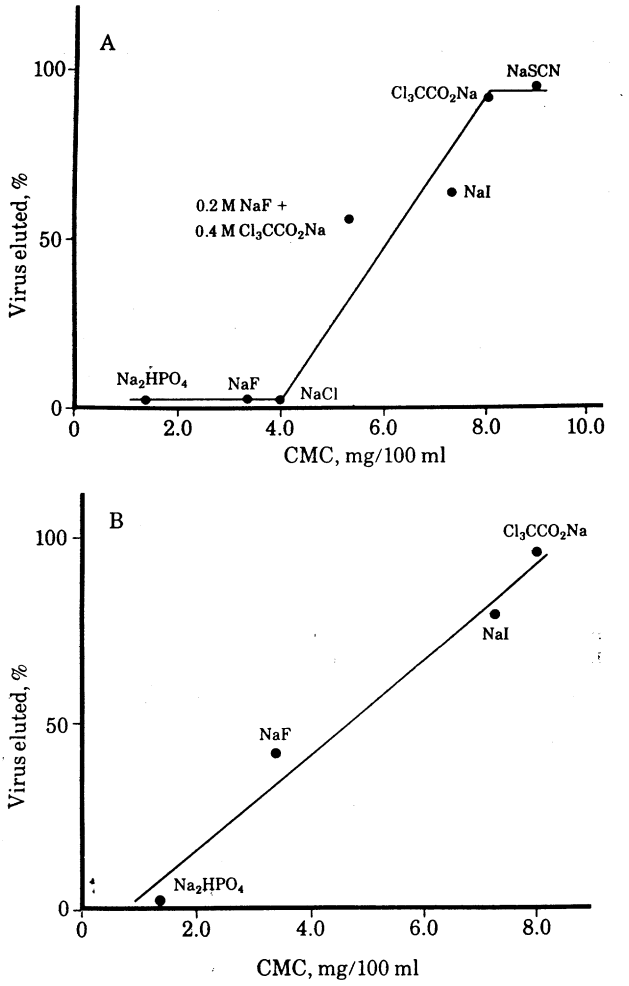

FIG. 1. Relationship of CMC to elution of viruses adsorbed on membrane filters. All salt solutions were in 0.02 M glycine (pH 9.5). The final concentration of the salt solutions was 0.6 M. (*A*) Elution from Zeta plus filters. (*B*) Elution from Millipore filters.

DISCUSSION

The ability of ions to influence the solubility of hydrophobic groups in different ways has been recognized for years (9, 11). Large, singly charged ions that tend to solubilize hydrophobic molecules are termed chaotropic ions. Examples of these are trichloroacetate and thiocyanate. In contrast, small, singly charged ions such as fluoride and multiply charged ions such as phosphate, sulfate, and citrate that decrease the solubility of hydrophobic molecules are called antichaotropic ions.

Kauzmann (12), Tanford (13), Ben-Naim (14), and others have proposed that hydrophobic interactions are a result of the unfavorable interaction of apolar groups with water rather than of attractions between different apolar groups. Consistent with this hypothesis, chaotropic agents are viewed as disordering the structure of water and thus reducing the thermodynamic barrier to the introduction of apolar groups to the aqueous environment (9, 15). Chaotropic ions have been used for the solubilization of membrane proteins (16) and to dissociate antigen–antibody complexes (17). By analogy, antichaotropic ions are thought to increase the structure of water (9, 15).

We have studied the effects of chaotropic and antichaotropic salts on the elution of viruses and compared them with the effects of these salts on micelle formation by a surfactant and on adenine solubility. Buffer alone and solutions of chaotropic salts were good eluents, had relatively high values for the CMC with Triton X-100, and increased the solubility of adenine. Solutions of antichaotropic salts prevented viral elution, had lower values for CMC, and decreased the solubility of adenine. The quantitative relationship between CMC and viral elution is shown in Fig. 1. At high pH, the ability to elute viruses adsorbed on membrane filters parallels the ability to accommodate hydrophobic functions.

At low pH (pH 4.0), neither solutions of chaotropic nor solutions of antichaotropic ions were effective in eluting viruses. At this pH, the virus and filter are oppositely charged (4, 5, 18) and the virus association with the membrane filter is likely maintained by electrostatic forces.

The difference in the abilities of chaotropic and antichaotropic ions to elute virus at pH 9.5 suggests that hydrophobic interactions are the major factor in virus–filter surface interactions at this pH. Both the virus and the membrane filters should be negatively charged at pH 9.5 (4, 5, 18). The electrostatic forces should therefore favor elution. Salt bridging by divalent cations or alterations of membrane charge by adsorbed ions have been considered as possible factors in maintaining virus–membrane associations under conditions where electrostatic effects would favor elution (4, 5, 18). These considerations have led to suggestions that chelating agents such as EDTA or citrate would be effective eluents for virus adsorbed on membrane filters (1, 5, 19). However, numerous attempts to elute

viruses by using these metal chelators have failed (6–8). In a recent study, Goyal *et al.* (20) found that EDTA often reduced the ability of 0.05 M glycine solutions to elute bacterial phage adsorbed to Zeta plus filters. The failure of metal chelators to act as eluents at high pH raises the question of the extent to which electrostatic interactions are involved in virus–membrane filter associations.

Our hypothesis that hydrophobic interactions, rather than salt bridges, are the dominant force stabilizing viral attachment to membrane filters at high pH is consistent with the failure of EDTA or citrate to elute viruses (6–8, 20). Both of these agents would be expected to act as antichaotrophs, stabilizing hydrophobic associations and preventing elution. With our buffer, both 3% EDTA and 3% citrate decreased the solubility of adenine (11% and 30%, respectively). Further, our hypothesis is consistent with the ability of detergents such as Tween 80 and sodium lauryl sulfate (1, 8) and uncharged molecules such as urea (7) to serve as eluents. In our buffer, 4 M urea was a potent chaotroph and caused a 34% increase in the solubility of adenine, consistent with previous reports of its chaotropic activity (15). Thus, it appears that, by using simple tests such as adenine solubility or CMC, the efficacy of solutions for viral elution can be predicted. Based on this relationship, it should be possible to design experimental conditions for both the binding and elution of viruses from field and laboratory samples. It seems likely that the extent of hydrophobic interactions with filters may vary for different types of viruses. Further, the filter materials currently available differ in their binding ability and others can be designed to provide wide ranges of surface properties. Thus, it should be possible to fractionate different types of viruses and to purify viruses by using adsorption-elution procedures and solutions of chaotropic and antichaotropic salts.

The technical assistance of O. Lanni is gratefully acknowledged. This work was supported in part by Grant R806290 (to S.R.F.) from the U.S. Environmental Protection Agency, Grant 1R01 GM 24059 (to L.O.I.) from the National Institutes of Health, Grant AA03816 (to L.O.I.) from the National Institute of Alcohol Abuse and Alcoholism, and the Florida Agricultural Experiment Station. L.O.I. is the recipient of Career Development Award K02 00036 from the National Institute of Alcohol Abuse and Alcoholism.

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