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Comparison of In Vitro and Cell-Mediated Alteration of a Human Rhinovirus and Its Inhibition by Sodium Dodecyl Sulfate[1]

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After human rhinovirus type 2 (HRV-2) attaches to HeLa cells, two types of subviral particles are formed which closely resemble particles produced in vitro by acid or heat. One type of particle contains RNA whereas the second sediments as an empty capsid and is RNA-deficient. Sodium dodecyl sulfate (SDS) at 10^{-4} M inhibits the cell-mediated formation of these particles from HRV-2 virions and the ability of HRV-2 to form plaques, but it does not inhibit the formation of plaques by human rhinovirus 14 (HRV-14). SDS also stabilizes HRV-2 against inactivation by acid or heat to a much greater extent than it does HRV-14. In a similar manner, SDS protects against the acid inactivation of the subpopulation of HRV-2 natural top component particles which attach to virus-specific cellular receptors. This suggests that the loss of native properties of natural top component particles and of virion are related processes. The basis for this alteration and also its role in infection are discussed.

The infectivity of human rhinovirus type 2 (HRV-2) is rapidly lost (eclipsed) after interaction of the virus with HeLa cells at 34.5 C (14). Cell-mediated loss of infectivity of both HRV-2 and coxsackievirus B3 produces particles which sediment about 10% more slowly than virions, lack the smallest virion polypeptide (VP4), contain viral RNA, but are noninfective because they do not attach to host cells (4, 5, 14). Poliovirus is also altered by cells to produce slowly sedimenting particles (8) which contain infectious RNA, but lack infectivity (11). In the cases of coxsackievirus and poliovirus, the altered particles which were studied had eluted from infected cells. However, analysis of the interaction of HRV-2 with HeLa cells indicated that the properties of the noninfectious particles which remained cell-associated resembled those of the smaller fraction of particles which eluted (14).

Until now, evidence has been lacking that alteration of cell-associated picornavirions represents a step on the infective pathway. Because the ratio of virus particles to PFU is several hundred to 1 for enteroviruses (4, 12), and about 2,000:1 for HRV-2 (12), it is conceivable that the smaller percentage of the particles causing infection behave differently from the major portion of virus particles. We have now found that a specific inhibitor, sodium dodecyl sulfate (SDS), prevents infection by HRV-2 and also inhibits the formation of subviral particles within the infected cell. This might be taken as evidence that the cell-mediated biochemical process observed after interaction of host cell with radioactive virus is a step on the pathway leading to the establishment of infection.

All rhinoviruses are inactivated by acid at pH 5 (16). In the case of HRV-2, this leads to the formation of particles (A-particles) which resemble, in their sedimentation behavior and polypeptide composition, the altered particles produced after interaction of HRV-2 with host cells (12, 17). A-particles lack infectivity because they are unable to attach to host cells (17). In addition to A-particles, a second class of more slowly sedimenting, RNA-free particles (B-particles) is also formed from HRV-2 virions by heat or other treatment (12, 15, 17). It will be shown that similar particles are formed in cells during the first hour of infection. We have found that SDS protects the infectivity of HRV-2 against acid or heat inactivation. This suggests that the in vitro alteration of virions can serve as a useful model for the alterations that occur in the infected cell.

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Both types of subviral particles derived from HRV-2 share certain heterotypically reactive immunodeterminants not found on native virions (15). The nature of the physical alteration of HRV-2 virions which exposes new antigenic determinants is not clearly established. Poliovirus and coxsackievirus show a similar antigenic alteration upon heat denaturation (13, 19, see literature reviewed in 15). It has been suggested that denatured poliovirus loses its native antigenicity, as well as the ability to attach to host cells, as a direct result of the loss of virion polypeptide 4 (VP4) (1, 2).

Noble and Lonberg-Holm (17) showed that a portion of the natural top component (NTC) particles produced during HRV-2 infection (HRV-2-NTC) can attach to specific cellular receptors and are lacking in detectable VP4. Such particles contain VP0, a large polypeptide; in the case of the NTC of poliovirus, VP0 has been shown to contain the amino acid sequences found in VP4 and VP2 (10). HRV-2-NTC has also been shown to contain some of the antigenic determinants of native virions, and to lose these upon acidification (15). We now present evidence that the ability of HRV-2-NTC particles to attach to cells is also lost upon acid treatment, and SDS protects against this loss. It is thus possible to view the protective effect of SDS on virions or NTC as a stabilization of a native protein conformation which is necessary for attachment to host cells.

MATERIALS AND METHODS

Virus and cells. The origin, propagation, and preparation of radioactively labeled HRV-2 and human rhinovirus type 14 (HRV-14) were described by Korant et al. (12). The preparation of purified, radioactive HRV-2-NTC has also been described (17).

Preparation of A- and B-particles in vitro. A-particles were produced from purified HRV-2 by mixing virus with an equal volume of 1.0 M sodium acetate buffer (pH 5.0). The mixture was left at room temperature for 20 min, chilled, and neutralized with 1 vol of 0.5 M Tris base (15). B-particles were prepared by heating purified virions in serum containing medium to 56 C for 5 min (15).

Incubation of virus and cells. HeLa cells were propagated in Spinner culture and washed before use as already described (14). These were mixed with purified virus, at 0 C, to give final concentrations of 10^7 cells/ml and 10^4 virion particles/cell. The mixture was then incubated in a shaking water bath at 34.5 C, and samples were removed at the times indicated. The cells were washed twice with 4 vol of ice-cold Spinner medium containing 5% heat-inactivated fetal calf serum (5% HIFC-Spinner medium), and resuspended in 0.02 M Tris-hydrochloride buffer (pH 8.1). Samples were assayed for cell-associated radioactivity and for cell-associated infectivity (after lysis with SDS), as previously described (14).

Isolation of subviral components from infected cells. Cells infected with radiolabeled HRV-2 were washed with medium and disrupted as already described (14). After addition of radioactive virions or virion-derived components as sedimentation markers, a sample of 1.0 to 1.2 ml of the disrupted cell suspension was analyzed by zonal centrifugation in 16-ml (⅝ by 4 inch) sucrose gradient containing 1 M NaCl (14). Gradients were centrifuged for 16 h at 15,000 rpm and 3 C in an SW27 rotor (Spinco Division, Beckman Instruments Co., Palo Alto, Calif.).

The gradients were fractionated dropwise from the bottom. The samples applied to the gradients contained 0.5% Nonidet P-40, causing a decrease in the drop size (and thus the fraction size) at the top of each gradient. Fractions of three drops were mixed with 0.6 ml of 0.5 M NaOH and were counted with 10 ml of Hayes solution number 3 (9) containing "Cabosil."

The fractions were counted in a scintillation spectrometer equipped with punched tape output. These data were analyzed by a "PPD 10" computer (Digital Equipment Corp., Maynard, Mass.), using a program written by B. Butterworth. The length of each gradient was normalized to unity.

Effect of SDS on the acid inactivation of HRV-2 and HRV-14. Concentrated pools of crude virus (10^9 to 3×10^9 PFU/ml) were clarified by low-speed centrifugation. Virus was mixed with an equal volume of a solution of SDS at twice the final concentration of SDS in 5% HIFC-Spinner medium. The virus mixture was then diluted 100-fold with 5% HIFC-Spinner medium buffered at various acidic pH values and containing SDS as indicated. The acidic medium had been first adjusted with acetic acid to pH 5.0, allowed to equilibrate, and subsequently adjusted with NaOH to the indicated pH. SDS was present both before and during acidification of virus. After 1 h at room temperature, samples of each diluted virus mixture were diluted 100-fold further into neutral medium and frozen for eventual infectivity assay.

Effect of SDS on heat inactivation of HRV-2 and HRV-14. Concentrated virus pools were diluted 200-fold into 5% HIFC-Spinner medium containing the desired concentration of SDS. Samples of 0.6 ml were incubated in a water bath at 50 C for various periods of time. After incubation, the samples were chilled to 0 C and frozen for eventual plaque assay of residual infectivity.

Acid inactivation of the ability of HRV-2 virions or NTC particles to attach to host cells. Purified HRV-2 virions or NTC particles in 5% HIFC-Spinner medium were diluted with an equal volume of the medium which contained no SDS or 60 μg of SDS per ml. The pH of such mixtures was adjusted by mixing 30 μliters of each with an equal volume of 1 M sodium acetate buffer at the pH values indicated in the figures. After 20 min at room temperature, the samples were chilled to 0 C, 500 μliters of 5% HIFC-Spinner medium was added, and the samples were neutralized with 0.5 M Tris base. Each sample was mixed with 550 μliters of HeLa cells at 2×10^7 cells/ml. Portions of 500 μliters of the cell-containing mixtures were incubated for either 0 or 15 min at 34.5 C and analyzed for the percentage of cell-associated radioactivity (17). The net uptake was normalized so that

virus or NTC diluted with one volume of 5% HIFC-Spinner medium (pH 7.2) and held 20 min at room temperature gave 100% cell association of radioactivity after addition of cells and incubation at 34.5 C for 15 min.

RESULTS

Alterations in virions after attachment to cell for 20 and 45 min. The major product of infection with HRV-2 virions after 15 min of interaction with HeLa cells at 34.5 C is a noninfective particle which contains RNA, sediments at 90 to 94% the rate of infectious virus (14), and resembles particles produced by acidification of virus (12). We will refer to particles with these properties as A-particles, regardless of whether they are produced in vivo or in vitro. Figure 1a shows the isolation of A-particles from cells infected for 20 min at 34.5 C with virus doubly labeled with ^{32}P-phosphate and ^{14}C-amino acids. After 20 min of incubation, 83% of the total added ^{14}C was cell associated. It has already been shown that, after 20 min, there is a net loss of radioactive label from cells infected under these conditions, as a result of the elution of noninfective A-particles (14). As shown in the experiment shown in Fig. 1, after 45 min of infection, only 65% of the total added ^{14}C was cell associated, i.e., 22% of the ^{14}C which was cell associated by 20 min had eluted by 45 min.

Figure 1b shows the sedimentation of the radioactive material which was cell associated at 45 min. There has been an increase in the amount of the more slowly sedimenting B-particles, which are deficient in or lack RNA. A careful comparison of the two parts of Fig. 1 indicated that there was a 70% increase in B-particles between 20 and 45 min of incubation. The radioactive profile in Fig. 1b also suggests that there may be particles which contain some RNA and have sedimentation rates intermediate between those of A- and B-particles.

The results in Fig. 1 and other experiments which are not shown suggest that A-particles are converted to B-particles during the first hour of infection at 34.5 C.

Comparison of particles produced in vivo and in vitro. Figure 2 compares the sedimentation of A-particles isolated from cells after 15 min of incubation with that of virions (Fig. 2a) and with that of A-particles produced by acidification of virus (Fig. 2b). A-particles produced in vivo and in vitro sediment together, provided that the comparison is made using particles isolated from cells after short periods of infection.

When A-particles produced by acidification of virus are cosedimented with particles obtained from cells 40 to 60 min after infection (for example, Fig. 2c), the latter particles exhibit a 4% slower sedimentation rate than the former. It was also observed that noninfectious A-particles eluting from cells after prolonged incubation sedimented slightly slower than A-particles produced by acidification of virus (data not shown).

In Fig. 2c the sedimentation of B-particles produced in vitro by heat is compared with the sedimentation of the RNA-deficient B-particles produced after 60 min of infection at 34.5 C. The major component present in the infected cells (B-particles) cosediments with the particles produced in vitro. A- and B-particles produced by a method of acidification employed in earlier work (12, 17) did not differ in sedimenta-

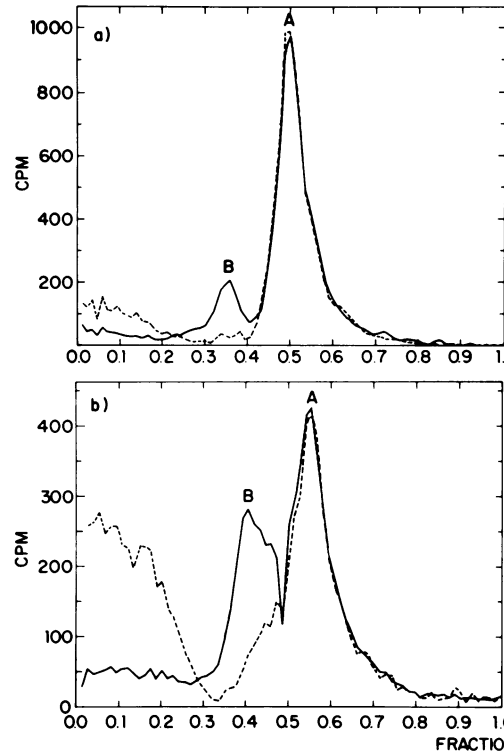

FIG. 1. *Subviral particles isolated after interaction of HRV-2 with HeLa cells. HRV-2 labeled with both ^{14}C-amino acids (——) and ^{32}P-phosphate (- - -) was used to infect cells at 34.5 C. The particles which were cell associated after 20 min (a) and 45 min (b) were isolated and analyzed by gradient centrifugation as described in Materials and Methods. The ^{32}P has been multiplied by 4.3 and corrected for decay during the time required for counting. The ratio ^{32}P/^{14}C of the original virus and in the A-particles was approximately 0.24 in each case. Less than 6% of the total radioactivity sedimented to the bottom of either gradient. Sedimentation is from left to right in this and all subsequent gradient profiles.*

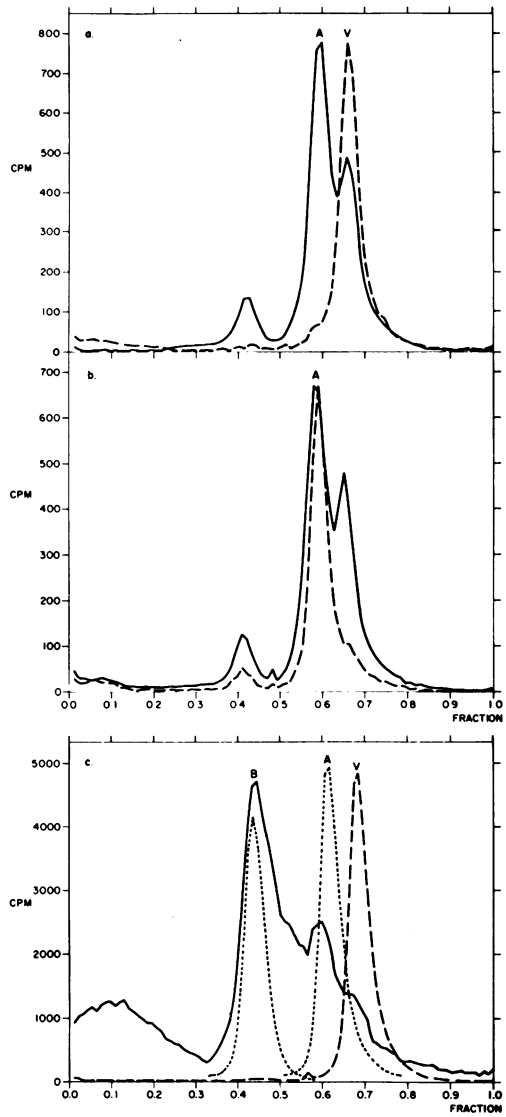

FIG. 2. *Comparative sedimentation of particles isolated from infected cells and particles produced in vitro. a, Cosedimentation of ^{3}H-labeled native HRV-2 (- - -) and ^{14}C-labeled particles (——) isolated from HeLa cells 15 min after infection at 34.5 C. b, Cosedimentation of ^{3}H-amino acid-labeled A-particles produced in vitro by acidification (- - -) and ^{14}C-amino acid-labeled particles isolated from cells after 15 min of infection (——). c, Profile of a gradient showing the sedimentation of ^{3}H-amino acid-labeled A-particles and B-particles produced in vitro (· · · · ·) and ^{3}H-labeled virus (- - -), relative to ^{14}C-amino acid-labeled, virus-derived material isolated from HeLa cells infected for 60 min at 34.5 C (——). The profile in part c is a composite from three gradients, each containing material from cells infected for 60 min and either ^{3}H-labeled A- or B-particles produced in vitro or ^{3}H-labeled virus. The ^{14}C-labeled peaks were superimposed to generate the composite profile.*

tion from A- and B-particles produced in vitro by the methods described in this report (data not shown).

Antiviral activity of SDS. We found that incorporation of 20 μg or more of SDS per ml in the agar overlay used in the plaque assay of HRV-2 greatly reduced or eliminated the ability of the virus to produce plaques, whereas 100 μg/ml was cytotoxic. This antiviral activity did not extend to HRV-14 or to poliovirus type 2, below concentrations which were cytotoxic (data not shown).

Inhibition of cell-mediated eclipse of HRV-2 by SDS. Subsequent investigation showed that SDS inhibited the cell-mediated eclipse of HRV-2 when present at levels comparable to those which inhibited plaque formation. Figure 3 illustrates the effect of SDS on cell-associated radioactivity and infectivity during the course of interaction of HRV-2 (Fig. 3a) and HRV-14 (Fig. 3b) with HeLa cells. At these low levels of SDS, the rate of attachment of virus to cells was essentially unaltered.

HRV-2 attaches efficiently to HeLa cells under the conditions employed, and the major portion of the viral radioactivity became cell associated in 7 min at 34.5 C. In the absence of SDS (top left panel), very little of the initial infectivity can be recovered from the cells after 45 min. The data represented in the lower panels on the left show that the eclipse of HRV-2 infectivity is drastically inhibited by 15 or 30 μg of SDS per ml.

The uptake of HRV-14 by HeLa cells is relatively slow (Fig. 3b). To detect the fate of cell-associated virus, the cells were washed free of nonadsorbed virus (approximately 90% of the original radioactivity) after 15 min of incubation and incubated again for 1 h after washing. In the absence of SDS (top right panel), there is extensive loss of cell-associated infectivity and also a significant loss of cell-associated radioactivity during incubation after washing. The loss of radioactivity is most probably due to elution of noninfective particles. From the lower panels in Fig. 3b it is evident that 15 or 30 μg of SDS per ml present before, during, and after washing does not greatly diminish either the loss of infectivity or elution during incubation.

Inhibition of the formation of cell-associated subviral particles by SDS. The inhi-

The data in panel c was obtained in a separate experiment from that depicted in panels a and b. For all the data presented in Fig. 2, the production of A- and B-particles in vitro and the isolation of cell-associated material and its analysis on sucrose gradients were carried out as described in Materials and Methods.

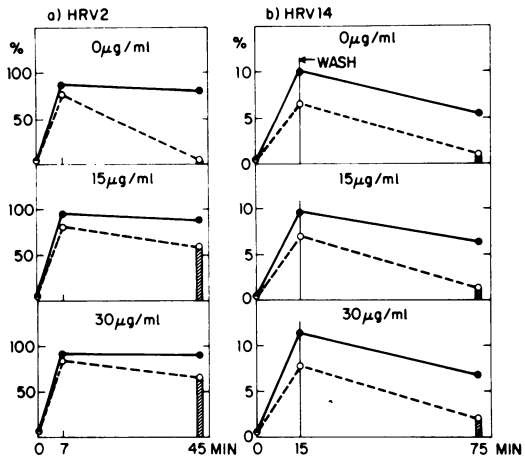

FIG. 3. *The absorption and eclipse of purified ^{14}C-amino acid-labeled HRV-2 (a) or HRV-14 (b) by HeLa cells at 34.5 C in the absence or presence of SDS. Cell-associated radioactivity (●——●) or infectivity (O- - -O) are expressed as the percentage of the total radioactivity or infectivity originally present in the suspension (at zero time), and the concentration of SDS present during all incubation periods is designated within each panel. In the panels on the right (b) the cells were washed twice after 15 min of incubation with HRV-14, using four volumes of medium containing the same concentration of SDS present during the incubation periods. The analysis of cell-associated radioactivity and infectivity were performed as described in Materials and Methods.*

bition of cell-mediated eclipse of HRV-2 infectivity by SDS (Fig. 3a) is correlated with the inhibition of the cell-mediated alteration of the virions by the detergent (Fig. 4). The presence of 30 μg of SDS per ml almost completely blocks the transformation of HRV-2 virions to A-particles during 45 min of incubation with HeLa cells at 34.5 C. Some B-particles were formed in the presence of SDS, although in a greatly reduced amount.

In vitro stabilization of HRV-2 infectivity by SDS. SDS specifically blocks HRV-2 eclipse, but not that of HRV-14 (Fig. 3a and b), which suggests that its site of action is the virion rather than the cell. This is supported by the following experiments.

In the first experiment (Fig. 5), HRV-2 and HRV-14 were incubated in acidified medium in the absence or presence of 20 μg of SDS per ml, as described in Materials and Methods. It can be seen in Fig. 5a that inactivation of HRV-2 by acid in the range of pH 5.2 to 5.4 is reduced about 1,000-fold in the presence of SDS. Figure 5b shows that HRV-14 is not as labile to acid as HRV-2 under these conditions and that SDS does not stabilize HRV-14 at either 20 μg/ml or 100 μg/ml.

Figure 6 illustrates the effect of 30 μg of SDS per ml on the inactivation of HRV-2 and HRV-14 at 50 C. Under the conditions employed (see Materials and Methods), SDS affords far greater protection to HRV-2 than to HRV-14. After 15 min of heating, the residual infectivity in HRV-2 is more than 1,000 times greater if the virus is heated in the presence of SDS, whereas the residual HRV-14 infectivity is only about four times greater if the virus is heated in SDS.

Inactivation of the ability of HRV-2-NTC to attach to HeLa cells. It has been reported that a portion of the HRV-2-NTC can attach specifically to HRV-2 receptors on host cells (17). The acid lability of the capacity of NTC to adsorb to cells was examined in the experiment shown in Fig. 7a. Purified virions or NTC particles were exposed to acetate buffer of various pH values for 20 min at room tempera-

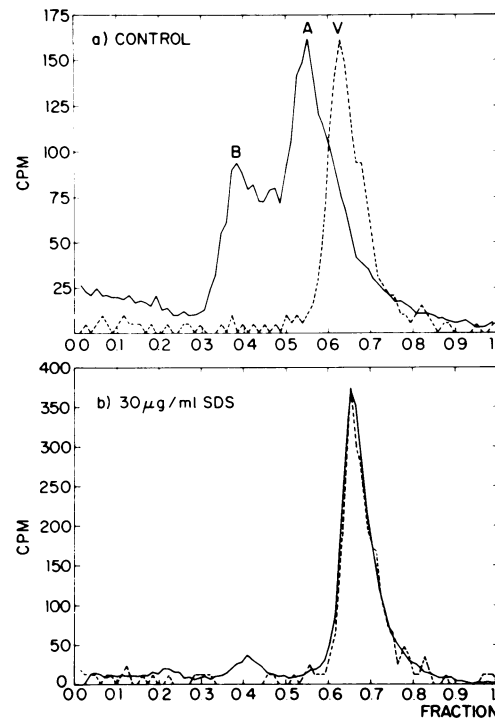

FIG. 4. *Inhibition by SDS of the formation of cell-associated subviral particles during infection of HeLa cells with HRV-2. HeLa cells were incubated with ^{14}C-amino acid-labeled HRV-2 for 45 min at 34.5 C in the absence (a) or presence (b) of 30 μg of SDS per ml. The infected cells were disrupted and analyzed for cell-associated, ^{14}C-labeled material on sucrose gradients as described in Materials and Methods, after addition of ^{32}P-labeled HRV-2 (- - -) as a sedimentation marker. Less than 6% of the ^{14}C-label applied to either gradient sedimented to the bottom.*

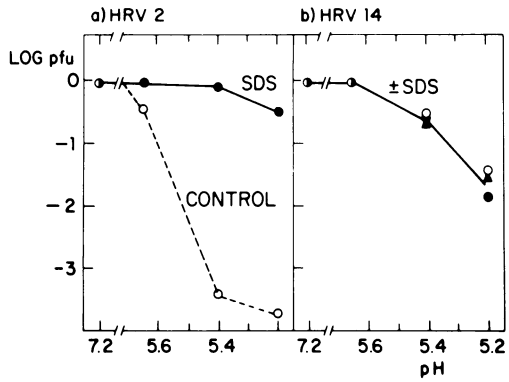

FIG. 5. *Selective stabilization of HRV-2 to acid inactivation. HRV-2 (a) or HRV-14 (b) were suspended in medium containing either no SDS (○) or 20 μg of SDS per ml (●), and diluted into acidified 5% HIFC-Spinner medium at the indicated pH (containing either 0 or 20 μg of SDS per ml) as described in Materials and Methods. After 1 h at room temperature, samples were diluted into neutral medium for infectivity assay. The infectivity of virus which had been incubated 1 h in neutral medium was expressed as 1.0 (log_{10} = 0). HRV-14 was treated in the same manner with 100 μg of SDS per ml (▲).*

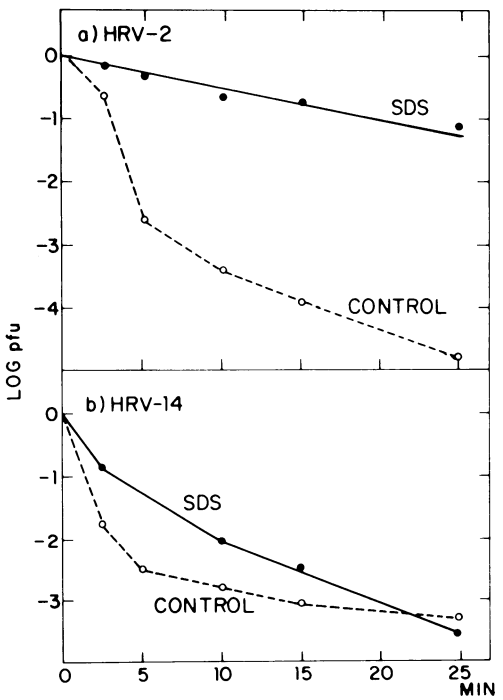

FIG. 6. *Selective stabilization of HRV-2 to heat inactivation. HRV-2 (a) or HRV-14 (b) was heated to 50 C in 5% HIFC-Spinner medium which contained no SDS (○- - -○) or 30 μg of SDS per ml (●——●) (see Materials and Methods). The residual infectivity in each sample was assayed and normalized to a starting infectivity of 1.0.*

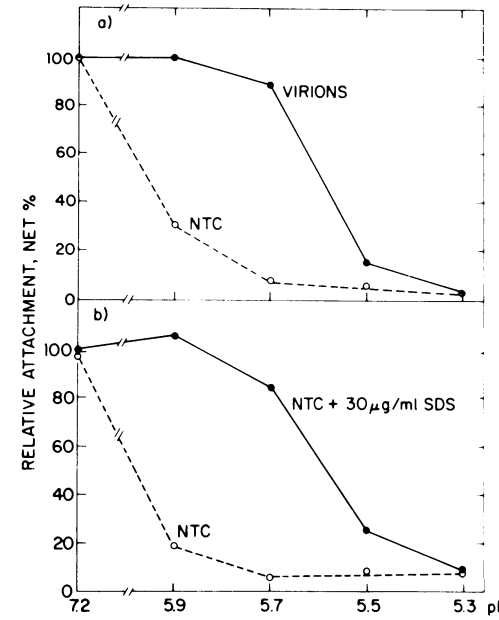

FIG. 7. *Acid inactivation of the ability of HRV-2 or HRV-2-NTC to attach to HeLa cells. Incubation of virions or NTC in acidified medium and subsequent treatment and analysis are described in Materials and Methods. The upper panel (a) shows the residual ability of ^{14}C-labeled virus (●——●) and ^{14}C-labeled NTC (○- - -○) to attach to cells after exposure to medium of various pH values. The lower panel (b) describes the residual ability of ^{14}C-labeled NTC to attach to cells after it is treated at various pH values in the absence of SDS (○- - -○) or in the presence of 30 μg of SDS per ml (●——●).*

ture and then diluted, neutralized, and incubated with cells for 15 min (see Materials and Methods). In this experiment, 91% of the unacidified virus and 32% of the unacidified NTC attached to the cells during incubation at 34.5 C. It can be seen that the ability of NTC particles to attach to host cells is much more labile to acidification than is that of intact virus. Virus inactivation occurs below pH 5.7, whereas most of the biological activity of NTC is lost at pH 5.9.

It was found, however, that SDS could stabilize NTC particles against the effects of acid (Fig. 7b). At 30 μg/ml, SDS stabilizes the ability of NTC to attach to host cells to the extent that the resultant pH stability curve resembles that of virus. Although not shown in Fig. 7b, the treatment of virus with pH 5.3 buffer in the presence of 30 μg of SDS per ml results in only a 5% inactivation of the capacity of the virus to attach to cells. According to these results, the ability of NTC particles to attach to host cells is less stable to acid treatment than that of native

virions, but it resembles the latter in being preserved by SDS. The purified NTC does not contain virus, as detected either by infectivity or by CsCl gradient equilibrium centrifugation (data not shown).

DISCUSSION

After 15 min of incubation of virus with cells, neither the cell-associated A-particles nor the cell-associated intact virions differed significantly in sedimentation from A-particles produced by acid or from purified virions, respectively (Fig. 2). Therefore, it is unlikely that these particles are associated with relatively large pieces of the cell membrane-receptor complex after isolation (which includes detergent treatment). There is indirect evidence that, in the absence of detergent treatment, HRV-2 A-particles (14) as well as eluted noninfective virions of poliovirus (8) and coxsackievirus (5) are associated with membrane fragments.

After longer periods of interaction with cells, the A-particles appear to sediment about 4% more slowly than the A-particles produced in vitro (Fig. 2c). This decrease is probably a result of either a minor conformational change or a loss or extrusion of a small portion of the RNA from the cell-associated A-particles.

The more slowly sedimenting B-particles, formed during incubation of virus with cells, are formed with complex kinetics, but must be largely derived from A-particles, especially after 45 min of incubation (Fig. 1). B-particles lack most or all of the virion RNA (Fig. 1), and sediment with RNA- and VP4-deficient particles which are produced by treatment of virions in vitro with heat (Fig. 2c). B-particles produced in vitro by acid (12) or by treatment with urea (15) sediment precisely with B-particles produced by heat (data not shown). All these particles have been assigned a 75 to 80*S* sedimentation coefficient relative to 150*S* HRV-2 virions. The conditions employed in gradient analysis of the cell-associated particles (see Materials and Methods) do not permit the accurate calculation of relative rates of sedimentation from the gradient profiles presented here.

The most likely model for the infective sequence is a transition from virions to A-particles, and from A-particles to B-particles by the loss of RNA. -Our view of the events following the attachment of HRV-2 virions to host cells is supported by an examination of the antiviral activity of SDS. SDS at low concentrations inhibited plaque formation and cell-mediated eclipse of HRV-2, but not of HRV-14 (Fig. 3). The formation of A- and B-particles during the eclipse of HRV-2 was also blocked (Fig. 4). If it were not for the specific antiviral effect, there would be little a priori reason to believe that either of these subviral particles lies in the infective pathway, because only about 0.05% of the virus particles lead ultimately to the production of detectable plaques in our assay (12). The blockade of infectivity and of the formation of subviral particles by SDS is strong circumstantial evidence that one or both classes of particles are a part of the infective process.

Some B-particles are found even at early times after infection (Fig. 1a), and it could be argued that A-particles do not lie on the infective pathway. However, the observation that a similarly small amount of B-particles is formed in the presence of inhibiting levels of SDS (Fig. 4) suggests that their formation alone is not sufficient for infection. Although B-particles are formed largely from A-particles, it is not certain that their production is necessary to initiate infection.

Since both the attachment of HRV-2 (14) and the formation of cell-associated subviral particles which appear to be necessary for infection (Fig. 1 and 4) are quite efficient, it seems likely that the relative inefficiency of the infectious process by HRV-2 (2,000:1, particles/PFU) must be attributed to a later step in the infectious process.

The in vitro inactivation of virions at pH 5 may be used as a model for the biological reactions leading to eclipse of HRV-2. The ease with which A-particles are produced by acid suggests that a similar proton-catalyzed process may occur in small regions (3) at or near the cell surface. We cannot at this time provide evidence for the mechanism of penetration of HRV-2 into the interior of the cell, but it may be relevant to note that acidified virions appear hydrophobic after protonation and are rapidly aggregated at pH 5 (17). This suggests the possibility that they might intercalate into the lipid portion of the membrane structure at the time and site of their formation.

A compound which can stabilize a virion against alteration may have antiviral activity. Eggers (7) has already found that rhodanine blocks infectivity of echovirus type 12 and also specifically stabilizes the virion against alkaline inactivation and against cell-mediated uncoating of the virion RNA. The discovery that SDS inhibits the acid and heat inactivation of HRV-2 in vitro at concentrations similar to those which inhibit cell-mediated eclipse (Fig. 5 and 6) proves that the site of action of SDS is the virion, as opposed to the cell membrane.

The ability of SDS to stabilize HRV-2 against inactivation in vitro shows considerable speci-

ficity (Fig. 5 and 6), in that HRV-14 is not similarly protected. In other respects, the action of SDS appears to be related to the well-known ability of this and other related detergents to bind to (18) and protect serum albumin against denaturation (6). We observed protection of HRV-2 with 10^{-4} M SDS (30 μg/ml). However, in our system, which contains both serum and sometimes cellular proteins, the concentrations of free SDS must be lower. We have detected significant binding of ^{35}S-labeled SDS to purified virions at approximately 10^{-5} M (unpublished data).

Since SDS protects HRV-2-NTC from acid inactivation (Fig. 7b), it must primarily stabilize HRV-2 protein-protein interactions, rather than protein-RNA interactions. The data of Fig. 7a show that the presence either of RNA or cleavage of VP0 to VP2 and VP4 in intact virions contributes additional stability against acid inactivation to the viral capsid.

The alteration in native virions which produces A- or B-particles in vitro also produces profound antigenic alteration (15). In accord with this, it was noted that preparations of purified HRV-2-NTC particles, a portion of which attaches to specific HRV-2 recepters, share some antigenic determinants with infectious virus, whereas acid-inactivated NTC particles do not (15). The biologically active population of NTC particles can be resolved from inactive particles, and it is the former population which shows native antigenicity (B. D. Korant, K. Lonberg-Holm, F. H. Yin, and J. Noble-Harvey, manuscript in preparation). Thus NTC particles capable of attaching to host cells are related to infectious virus in that the biological activity of both requires similar surface structure, i.e., antigenic composition, and both are stabilized by SDS.

Since VP2 and VP4 were not detected in biologically active or inactive HRV-2-NTC particles (17), it seems likely that the lack of biological activity of A- or B-particles also does not result primarily from their lack of VP4. It should be noted that this is not entirely conclusive because it is possible that biologically active HRV-2-NTC particles contain a few undetected molecules of cleaved VP0 to which their activity may be attributed. We consider it more likely that the native properties of both virions and of biologically active HRV-2-NTC particles depend upon a metastable conformation of the virion polypeptides, and that irreversible alteration of this conformation leads to loss of their ability to recognize cellular receptors. This alteration occurs after virions have attached to host cells and may be necessary to permit the subsequent movement of A-particles into the cell or for the uncoating of the virion genome, or both.

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