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Pathway of Rhinovirus Disruption by Soluble Intercellular Adhesion Molecule 1 (ICAM-1): an Intermediate in Which ICAM-1 Is Bound and RNA Is Released

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We have examined the pathway of rhinovirus interaction with soluble intercellular adhesion molecule 1 (sICAM-1). Binding of sICAM-1 to rhinovirus serotypes 3 and 14 gives particles with sedimentation coefficients from 145 to 120S, depending on the amount of sICAM-1 bound. The formation of 120S particles is faster and more extensive at a neutral pH than at an acidic pH. A large number of receptors (>30) can bind to human rhinovirus 3 without disruption. Disruption by sICAM-1 of rhinovirus that yields 80S particles is strongly temperature dependent and is antagonized by a low pH. Interestingly, sICAM-1 remains bound to the viral capsid after RNA is released, although in smaller amounts than those observed for the native virus. We have found heterogeneity both between and within 80S particle preparations in the VP4 content and number of bound receptors. The ability of the virus to remain bound to its receptor during the uncoating process may facilitate the transport of the viral genome into the cytoplasm in vivo.

Human rhinoviruses (HRVs) are small nonenveloped RNA viruses of the picornavirus family that cause 40 to 50% of all cases of common colds (26). Determination of the structure of two HRVs revealed that these viruses have a capsid of icosahedral symmetry and are 300 Å (30 nm) in diameter (10, 21). The outer part of the proteinaceous capsid is constructed from 60 copies of the viral coat proteins VP1, VP2, and VP3. The viral genome and VP4 are located inside the proteinaceous coat. A surface depression, or canyon, encircling each of the 12 fivefold vertices was proposed as the receptor-binding site (20). This hypothesis has been confirmed by structural and mutational analysis (1a, 19).

Intercellular adhesion molecule 1 (ICAM-1) is the receptor for the major group of rhinoviruses (5, 30, 31). ICAM-1 is a membrane protein with five immunoglobulin (Ig)-like extracellular domains, a hydrophobic transmembrane domain, and a short cytoplasmic domain (25, 29). The binding site for the virus is located in the N-terminal domains 1 and 2 (27). A recombinant soluble form of ICAM-1 (sICAM-1) truncated at the membrane that contains all five Ig-like domains has been shown to inhibit rhinovirus infection (14) and induce irreversible modification of the viral capsid in vitro (6, 8, 17). High efficiency in rhinovirus neutralization has been obtained with multivalent ICAM-1 Ig chimeras (17).

After binding of rhinovirus and other picornaviruses to the receptor on the cell membrane, the viral RNA is released from the capsid (uncoating) and delivered to the cytoplasm (22). It has been proposed that penetration of picornaviruses into the cell involves conformational changes in the viral particle that increase the hydrophobicity of the capsid, promoting the binding of the viral capsid to the lipid bilayer and the penetration of the lipid bilayer by viral RNA (3, 15). Lysosomal acidification and receptor binding have both been proposed to be important in inducing changes in the viral capsid that lead to productive infection (8, 9, 12, 13).

Hoover-Litty and Greve (8) showed that a particle of 135S and another of 80S that lacked VP4 and RNA were formed upon interaction of sICAM-1 with the virus. They found in studies with HRV-14 and -16 that the 135S particle could contain between 40 and 60 sICAM-1 molecules per virus and that the 80S particle contained no bound sICAM-1. Another particle with a sedimentation rate of 135S lacking VP4 was formed by acidification of the virus. Hoover-Litty and Greve showed that the uncoating rate is dependent on rhinovirus serotype and temperature and that a low pH (5.0) and sICAM-1 were not additive in promoting formation of the 80S particle. They proposed that RNA uncoating in vivo can be mediated by the receptor, endosomal acidification, or both and that different serotypes may vary in their strategies for delivery of the viral genome into the host cell.

We have studied the interaction of sICAM-1 with rhinovirus in solution to obtain information about the pathway of the uncoating mechanism. We have found that particles are formed with sedimentation coefficients between 145 and 120S, depending on the amount of sICAM-1 bound. We have extended previous studies on the effects of pH and temperature on the virus-receptor interaction and on the composition of the 80S particles that are formed when HRV is disrupted. Interestingly, we have found that sICAM-1 remains bound to the capsid after RNA uncoating. There is heterogeneity among 80S particles that may reflect a continuum in the disruption process. We propose that the ability of the virus to remain bound to the receptor during the uncoating process could facilitate the interaction of the capsid with the membrane and the transport of the viral genome into the cytoplasm in vivo.

MATERIALS AND METHODS

sICAM-1. A recombinant baculovirus containing the mutant cDNA clone (Y452E/F*) that codes for sICAM-1 containing the five extracellular Ig-like domains of the molecule was used to produce the protein (2). SF9 cells infected with the recombinant baculovirus secreted sICAM-1 into the media at up to 20 μg/ml. Protein was purified by immunoaffinity chromatography on ICAM-1 monoclonal antibody (R6.5) Sepharose. Protein eluted from the column was dialyzed against phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 1.47

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mM KH_2PO_4, 4.86 mM Na_2HPO_4, 0.68 mM $CaCl_2$, and 0.49 mM $MgCl_2$; pH 7.4). An extinction coefficient of 0.8 ml/mg · cm was used to calculate protein concentration, based on the amino acid composition of sICAM-1. The molecular weight (49,610) for the unglycosylated protein was used to calculate molarity.

To metabolically label sICAM-1, insect cells infected with recombinant baculovirus (multiplicity of infection, 10.0) were incubated for 2 days in cysteine- and methionine-free media containing a total of 0.1 mCi of [^{35}S]cysteine and [^{35}S]methionine per ml. Labeled sICAM-1 was purified from the media and protein concentration was determined as described above.

Virus. Original HRV-3 and HRV-14 stocks were obtained from the American Type Culture Collection and Roland Rueckert (University of Wisconsin), respectively. Virus was propagated in HeLa H1 cells. An HRV clone was plaque purified and used to obtain concentrated viral stock and purified virus as described by Rueckert and Pallansch (23). To eliminate the sucrose, virus was pelleted by ultracentrifugation from diluted viral stock and resuspended in PBS–0.01% bovine serum albumin (BSA). Sedimentation of the virus was carried out for 3 h at 40,000 rpm at 4°C in a Beckman SW55 rotor. Virus concentration was calculated from optical density at 260 nm (1 unit = 9.4×10^{12} virus particles per ml) (23). Plaque-forming assay of the viral stock was performed as described elsewhere (24). About 600 virions per PFU was determined for the purified virus stock.

Virus was radiolabeled as described elsewhere (23). For RNA labeling, 0.5 mCi of [5-^{3}H]uridine (27 Ci/mmol) was added to 37.5 ml of cells at 4×10^6/ml in minimal essential medium. For amino acid labeling, 0.5 mCi of L-[4,5-^{3}H]leucine (150 Ci/mmol), 0.5 mCi of [^{35}S]methionine (1,200 Ci/mmol), or 0.5 mCi total of [^{35}S]methionine and [^{35}S]cysteine (1,200 Ci/mmol) was added to 25 ml of cells at 4×10^6/ml in minimal essential medium without amino acids.

Sucrose gradient sedimentation. Gradients with 5 ml of 5 to 30% sucrose were used to analyze the disruption of the rhinovirus capsid. [^{3}H]leucine-labeled HRV-3 in PBS-BSA (15,000 cpm in about 5 μl) was diluted to a final concentration of 2.6×10^8 virus particles per μl in 50 μl of PBS or PIPES buffer (100 mM NaCl, 20 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], 1 mM $CaCl_2$, 1 mM $MgCl_2$)–5% fetal calf serum (FCS) with the indicated concentration of sICAM-1 and incubated at the indicated temperature, pH, and time. After incubation, samples were chilled on ice for 2 min, layered over a 5-ml linear 5 to 30% sucrose gradient in PBS-BSA, and centrifuged for 1.5 h at 40,000 rpm at 4°C in a Beckman SW55 rotor. Fractions (200 μl for fractions 1 to 18; 600 μl for fractions 19 and 20) were collected from the bottom by inserting a microcapillary and subjected to scintillation counting after addition of 2 ml of Scintiverse II (Fisher Scientific Co.). The percentage of total counts per minute was calculated for each fraction.

In some experiments, the decrease in percent counts per minute in the 150S region or in the region including the 150S and ~120S peaks with respect to the value for control virus was determined (see Fig. 1). The decrease in the latter region was used to calculate percent disruption, i.e., conversion to 80S particles. Percent decrease or disruption (in counts per minute) was calculated as (control − experimental)/control × 100.

To determine accurately the sedimentation coefficient of the native virus and other viral particles, linear 5-ml 5 to 20% sucrose gradients in PBS-BSA were used. Migration that is linear with the sedimentation coefficient of biological macromolecules in these sucrose gradients has been reported (16). To calculate the sedimentation of the particles that result from the interaction of sICAM-1 with HRV-3, we used HRV-14, which has a sedimentation coefficient of 150S (11) as a standard. [^{35}S]methionine-labeled HRV-3 was treated with different sICAM-1 concentrations in PIPES buffer (pH 7.0)–5% FCS for 30 min at 37°C. The samples (50 μl) were chilled on ice and mixed with [^{3}H]leucine-labeled HRV-14. This mix was layered on the top of a 5-ml linear 5 to 20% sucrose gradient and subjected to ultracentrifugation for 1 h at 40,000 rpm at 4°C in a Beckman SW55 rotor. Fractions were collected from the bottom as described above, and ^{3}H and ^{35}S scintillation was counted to determine the positions of the HRV-3 and HRV-14 particles (data not shown). The sedimentation coefficient of the HRV-3 natural empty capsid was also determined by this methodology.

Gradients with 1 ml of 5 to 30% sucrose were used to study cosedimentation of sICAM-1 with rhinovirus. To examine the cosedimentation of sICAM-1 with rhinovirus, [^{35}S]Met- and [^{35}S]Cys-labeled sICAM-1 (20,000 cpm; final concentration, 2.0 μM) was incubated with HRV-14 (1.4×10^{10} virus particles per μl) or HRV-3 (9.4×10^9 virus particles per μl) in PBS–5% FCS for 15 min at 37°C. Samples (25 μl) were chilled on ice and sedimented through a 1-ml linear 5 to 30% sucrose gradient for 1 h at 40,000 rpm at 4°C in a Beckman SW55 rotor. After centrifugation, tubes were stuck with a needle at the bottom and drops were collected as individual fractions (50 μl). A 45-μl aliquot of each fraction was scintillation counted. Samples containing untreated [^{35}S]methionine-labeled HRV-3 or HRV-3 treated with unlabeled sICAM-1 were processed in parallel.

To obtain virus-receptor complex for electrophoresis, [^{35}S]methionine-labeled HRV-3 (100,000 cpm) was treated with [^{35}S]Met- and [^{35}S]Cys-labeled sICAM-1 in PIPES buffer (pH 7.0) containing 5% FCS. Samples (25 μl) with sICAM-1-treated virus or virus alone were layered on 1-ml 5 to 30% sucrose gradient and centrifuged, and fractions were collected as described above. Aliquots of 10 μl were scintillation counted, and aliquots of 40 μl were subjected to sodium dodecyl sulfate (SDS)–10% polyacrylamide gel electrophoresis (PAGE) under reducing conditions and fluorography with En^{3}Hance (DuPont).

Bands corresponding to sICAM-1 and VP0 to -4 were cut from the dried gel after location by autoradiography, and radioactivity contained in the bands was determined by scintillation counting of the gel fragments with 2 ml of Scintiverse II (Fisher). The number of counts per minute calculated as background in each experiment was deduced from the counts per minute obtained for the electrophoretic bands. An electrophoretic band coming from the viral proteins that migrated close to the receptor increased the background in the determination of the counts per minute for the receptor. Experiments with labeled virus and unlabeled receptor showed that this band was 0.9% of the VP0 to -3 counts per minute in the ~120S particle and 3% of the VP0 to -3 counts per minute in the 80S particle. The estimated number of counts per minute for this band was deduced from the counts per minute obtained for sICAM-1 in each fraction.

The numbers of sICAM-1 molecules and virus particles in each gradient fraction were calculated by subjecting known amounts of [^{35}S]methionine-labeled HRV-3 and [^{35}S]methionine- and [^{35}S]cysteine-labeled sICAM-1 to SDS-PAGE and scintillation counting as described above. Standard curves were constructed with radioactivity of viral protein (VP0 to -3) and receptor electrophoretic bands plotted versus the corresponding numbers of viral particles and sICAM-1 molecules loaded in the gel.

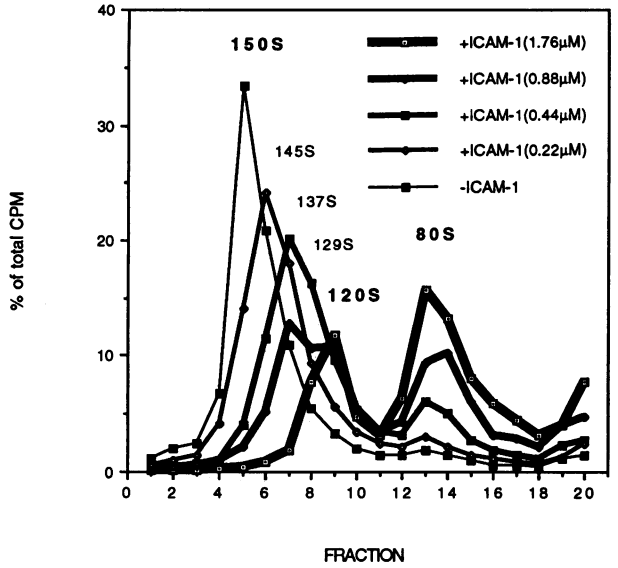

FIG. 1. Effect of sICAM-1 on virus sedimentation. [^{3}H]leucine-labeled HRV-3 was incubated with the indicated concentration of sICAM-1 for 30 min at 37°C in PIPES buffer (pH 7.0)–5% FCS and sedimented through a 5 to 30% sucrose gradient.

Determination of the activation energy for rhinovirus disruption. The sICAM-1-dependent disruption (dis) can be simplified as $V + n_I \rightleftharpoons Vn_I \xrightarrow{k} \mathrm{dis}Vn_I$, where V is the amount of virus, n_I is the number of bound ICAM-1 molecules, and k is the kinetic rate constant. Since formation of Vn_I occurred rapidly with respect to disruption, we will ignore the first part of this reaction and treat V below as virus with an equilibrium number n of ICAM-1 molecules bound. We have found little change in the affinity of sICAM-1 for rhinovirus from 10 to 25°C using surface plasmon resonance spectroscopy (unpublished data).

We can express the velocity of disruption as a decrease in intact viral particles with time (t): $-dV/dt = kV$. Integrating, $\ln(V_0/V) = kt$.

V_0 and V represent the initial and final concentrations of nondisrupted virus, respectively. Then, substituting k into the Arrhenius equation, we have $\ln(V_0/V) = tAe^{-E_a/RT}$, where E_a is the activation energy, A and R are the Arrhenius and gas constants, respectively, and T is the temperature in kelvins. If we obtain logarithms from both terms of the equation we have $\ln[\ln(V_0/V)] = \ln(tA) - E_a/RT$. Compared with percent disruption (D), $V_0/V = [100/(100 - D)]$.

RESULTS

Disruption of the rhinovirus capsid by sICAM-1. HRV-3 was incubated for 30 min at 37°C with different concentrations of sICAM-1, a soluble, truncated molecule containing the entire extracellular domain with all five Ig-like domains, and subjected to sucrose gradient sedimentation (Fig. 1). Increasing concentrations of sICAM-1 caused the native virus (150S) to progressively sediment more slowly (145 to 120S), suggesting that increased numbers of sICAM-1 molecules bound per virion slowed sedimentation. At increasing sICAM-1 concentrations, the amount of virus sedimenting at 150 to 120S decreased and particles sedimenting at 80S appeared, along with some material at the top of the gradient. Disrupted HRV-3 particles sedimented more slowly than HRV-3 natural empty capsids (~90S). The 145 to 120S particles contained identical amounts of RNA and VP1 to -4 and were infective. The 80S particle had lost RNA and infectivity (data not shown).

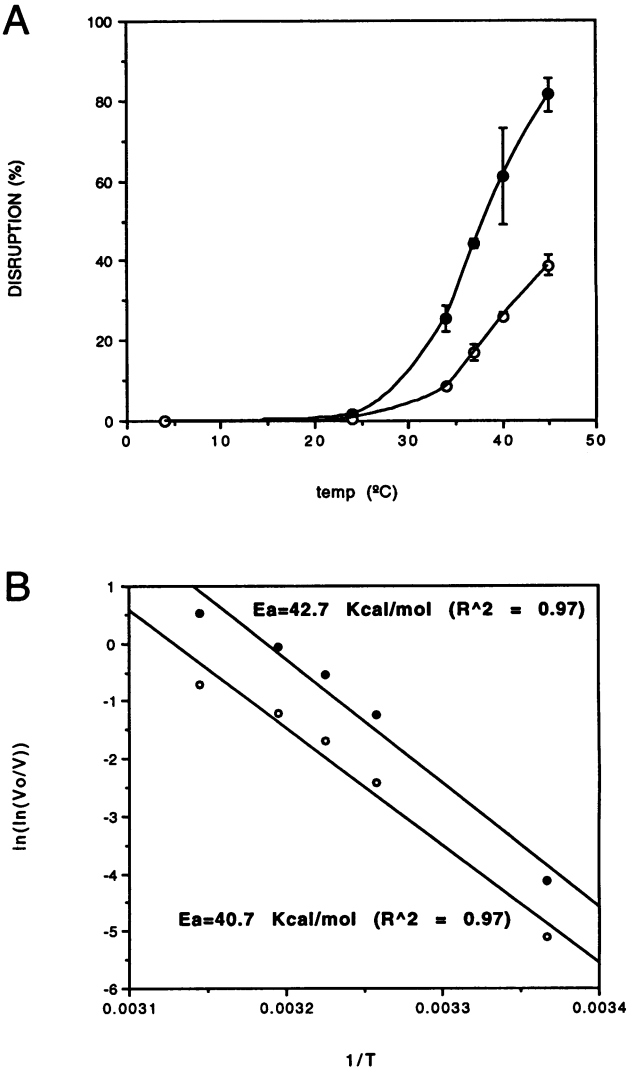

FIG. 2. Effect of temperature on rhinovirus disruption and determination of the activation energy for disruption. [^{3}H]leucine-labeled HRV-3 (2.6×10^8 virus particles per μl) was incubated for 30 min at different temperatures without (open circles) or with (closed circles) sICAM-1 (0.44 μM) as in Fig. 1. After treatment, samples were subjected to sucrose gradient sedimentation and percent disruption was calculated as reduction of the 150 and ~120S peaks. (A) Disruption as a function of the temperature. The data are averages for two different experiments. (B) Arrhenius plot of the data in panel A. The activation energy was obtained from the slope of the curve obtained by linear regression.

Temperature and disruption. Disruption increased exponentially with temperature above 30°C (Fig. 2A). We have found no change in the affinity of sICAM-1 for rhinovirus with changes in temperature using plasmon resonance spectroscopy (1). Using the Arrhenius equation, $k = Ae^{-E_a/RT}$, we found that ln k was linearly related to $1/T$ (Fig. 2B) and the energy of activation for disruption was about 42 kcal (ca. 176 kJ)/mol of virion. The activation energy for disruption did not change

when sICAM-1 was bound; rather, the effect of sICAM-1 was to accelerate disruption at a given temperature and to lower the temperature at which a given rate of disruption occurred by 5°C.

pH and disruption. Like other picornaviruses, rhinovirus shows low stability at a moderately acid pH (<6.0) (22). We found that a low pH is not synergistic with ICAM-1 in promoting disruption of the viral capsid. The presence of sICAM-1 increased capsid disruption at pH 7.0 and 6.5 but not at pH 6.0 or 5.6 (Fig. 3A). When the amounts of disruption in the presence and absence of ICAM-1 were compared, the disruption dependent on ICAM-1 diminished with decreasing pH over a wide range of ICAM-1 concentrations (Fig. 3B). We found that both the rate and the extent of formation of ~120S particles at 37 and 24°C were greatly diminished at pH 6 compared with those at pH 7 (Fig. 3C). Thus, the decrease in the sICAM-1-dependent disruption reflected the considerably lower binding of sICAM-1 to rhinovirus at a lower pH. Studies with plasmon resonance spectroscopy have confirmed that the binding of sICAM-1 to rhinovirus is lower at pH 6 than at pH 7 (1).

Cosedimentation of sICAM-1 with the viral particles. To examine ICAM-1 binding to the nondisrupted 150 to 120S and the disrupted 80S viral particles, HRV-3 and HRV-14 were incubated with [^{35}S]methionine- and [^{35}S]cysteine-labeled sICAM-1 and subjected to sucrose gradient sedimentation (Fig. 4). In the absence of the virus, sICAM-1 remained at the top of the gradient (data not shown). After incubation with HRV-3 (Fig. 4A) and HRV-14 (Fig. 4B), a portion of the sICAM-1 cosedimented with both the 120S and the 80S viral particles. Peaks of sICAM-1 were clearly associated with each class of viral particle. Preincubation of sICAM-1 with R6.5 antibody, which blocks binding of ICAM-1 to rhinovirus (27), abolished cosedimentation of sICAM-1 (data not shown).

The ratio of sICAM-1 to virus and the capsid composition of the viral particles were examined by coincubating HRV-3 and sICAM-1 that were both radiolabeled with ^{35}S and subjecting them to sedimentation (Fig. 5A), SDS-PAGE (Fig. 5B), and scintillation counting of specific bands (Fig. 6). sICAM-1 clearly cosedimented with intact virions in fractions 5 to 8 (Fig. 5B); however, there was a slight difference in peak positions. The peaks of virion proteins and sICAM-1 were in fractions 6 and 7, respectively (Fig. 5B and 6A and B).

The number of sICAM-1 molecules bound per virion varied from fraction 5 to 8 (Fig. 6C). This likely reflected heterogeneity in the starting population in the number of sICAM-1 molecules bound per virion and, perhaps, some dissociation of sICAM-1 from the virions during sedimentation. Virions with less bound ICAM-1 sedimented more rapidly. Fraction 8 (~120S) contained the highest number of sICAM-1 molecules per virion, 25.5 in this experiment (Table 1). In other experiments, the number of sICAM-1 molecules bound per virion in the ~120S fractions increased with increasing concentrations of sICAM-1 (Table 1).

A second peak of sICAM-1 consistently cosedimented with the 80S empty-capsid peak (fractions 10 and 11 [Fig. 5B and 6B]). The number of sICAM-1 molecules per empty-capsid particle was 22 for these fractions in experiment 1 (Table 1). An experiment in which the percentage of disrupted virions (80S particle) was higher with the same viral stock gave lower sICAM-1/empty-capsid ratios (6.0). With a second virus stock, the same trend was seen. The number of sICAM-1 molecules bound per empty virion was highest when the percent disruption was moderate and decreased as the percentage of disrupted virions increased (Table 1).

There was a similar trend for the content of VP4 in the empty virions. Quantitated as a percentage of capsid protein VP0 to -3 radioactivity, the content of VP4 in the 120S particles was similar to that in the native virus (Fig. 6D; Table 1). In experiment 1 in Fig. 6, 64% of the VP4 was released from the empty capsids. There was variation between the two different viral stocks, but within each stock there was greater

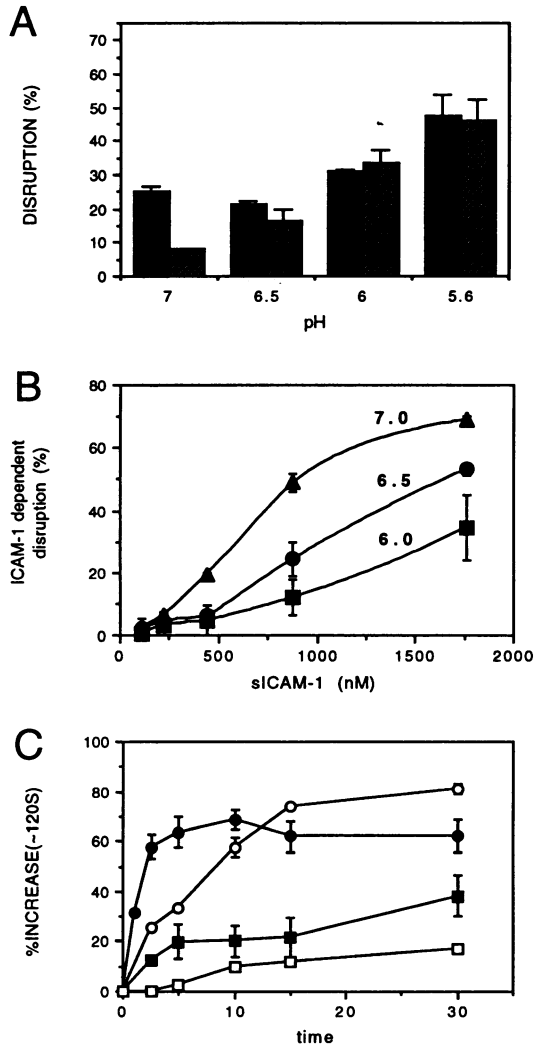

FIG. 3. Effect of pH on sICAM-1 binding to rhinovirus and viral disruption. (A) [^{3}H]leucine-labeled HRV-3 with or without sICAM-1 was incubated for 30 min at 37°C in PIPES buffer at the indicated pH with 5% FCS. Disruption in the presence of 0.44 μM sICAM-1 (solid bars) or absence of sICAM-1 (hatched bars) was determined as described in Materials and Methods. The data are averages and ranges for two different experiments. (B) HRV-3 was incubated as described for panel A at pH 7.0 (▲), 6.5 (●), or 6.0 (■) without sICAM-1 or with the indicated concentrations of sICAM-1. Disruption dependent on ICAM-1 was calculated as disruption in the presence of sICAM-1 minus disruption in the absence of sICAM-1. The data are averages and ranges (bars) for two experiments. (C) HRV-3 was incubated as described for panel A for the indicated time at 37°C (closed symbols) or 24°C (open symbols) either at pH 7.0 (circles) or at pH 6.0 (squares). % Increase (~120S), difference between the decrease of 150S particles (i.e., formation of ~120 and 80S particles) and decrease of 150 and ~120S particles (i.e., formation of 80S particles). The data are averages and standard deviations (bars) for two or three experiments.

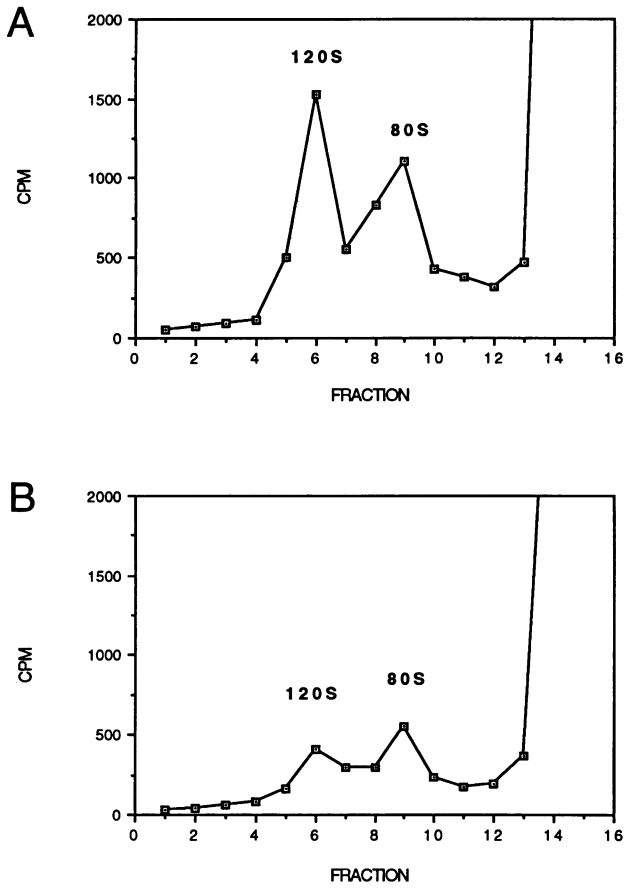

FIG. 4. Cosedimentation of sICAM-1 with HRV-3 and HRV-14 120 and 80S particles. HRV-3 (A) and HRV-14 (B) were incubated with [^{35}S]Met- and [^{35}S]Cys-labeled sICAM-1 and subjected to sucrose gradient sedimentation. [^{35}S]methionine-labeled HRV-3 treated with or without unlabeled sICAM-1 was sedimented in parallel to define positions of the 150, 120, and 80S peaks.

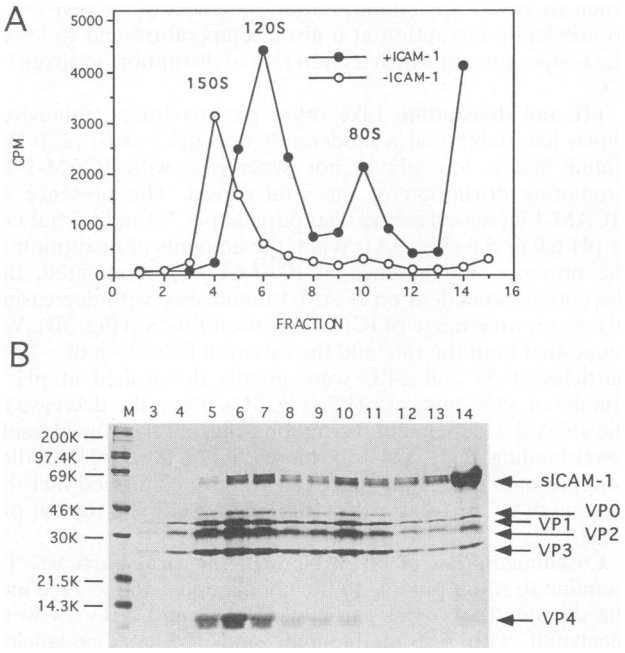

FIG. 5. Sedimentation of radiolabeled HRV-3 and sICAM-1. [^{35}S]methionine-labeled HRV-3 was incubated with or without [^{35}S]methionine- and [^{35}S]cysteine-labeled sICAM-1 (1.1 μM) for 20 min at 37°C in PIPES buffer (pH 7)–5% FCS and subjected to sucrose gradient sedimentation. Aliquots of fractions were scintillation counted (A) or subjected to electrophoresis and fluorography (B). The data in panels A and B are from the same experiment.

loss of VP4 from the 80S empty-capsid fraction when the overall percentage of disrupted virions was higher (Table 1).

DISCUSSION

We have examined the pathway of rhinovirus interaction with sICAM-1, analyzed biochemically the virus-receptor complexes, and determined the effects of pH and temperature on the virus-receptor interaction. Binding of sICAM-1 to rhinovirus first resulted in formation of a particle with a sedimentation coefficient that varied from 145 to 120S, depending on the amount of sICAM-1 bound per virion. The infectivity and content of viral capsid proteins and RNA of this particle were similar to those of the native virus (6). At higher temperatures and higher concentrations of sICAM-1, the soluble receptor was able to induce disruption of the virus. The disrupted particle lacked RNA, had a lower VP4 content, was noninfectious, and sedimented at 80S. We found that disrupted virions continued to bind sICAM-1. Binding was highest when the overall amount of viral disruption was moderate and when release of VP4 from the disrupted virions was not maximal, suggesting that we may have observed an intermediate stage in the disruption process. A low pH also induced virus disruption but was antagonistic to binding of the receptor and receptor-dependent viral disruption.

The increased mass of particles with bound sICAM-1 would tend to increase sedimentation. sICAM-1 has an extended, rod-like shape and a high frictional coefficient (27). The decrease in sedimentation, therefore, appears to be due to an increased frictional coefficient. An expansion of the capsid related to the interaction with the receptor could also explain this effect; however, electron microscopy of a two-domain fragment of ICAM-1 bound to rhinovirus suggested binding to the canyon around the fivefold axis in the virion and revealed no conformational changes in the virion (19). We found that up to 25 to 44 sICAM-1 molecules per HRV-3 120S particle could be bound at 37°C. Binding of the theoretical maximum of 60 receptors per virus has been reported for other rhinovirus serotypes (8).

Both a mildly acidic pH and binding to receptors have been shown to induce conformational changes in rhinovirus and poliovirus capsids in vitro (6, 9, 11, 22). We tested whether these two factors were synergistic. As the pH was decreased below pH 7, the amount of capsid disruption in the absence of the receptor increased and the amount of additional disruption in the presence of the receptor decreased. Thus, there is no synergy between receptor-mediated disruption and pH-mediated disruption. The lesser effect of sICAM-1 at a lower pH could be completely explained by our finding that it associates with rhinovirus to a lesser extent at pH 6, implying lower affinity. Direct measurements with surface plasmon resonance showed that the affinity is decreased at pH 6 compared with pH 7 (1). Whether lysosomal acidification or receptor-induced conformational changes are important for RNA uncoating of picornaviruses in vivo is still unknown, and controversial

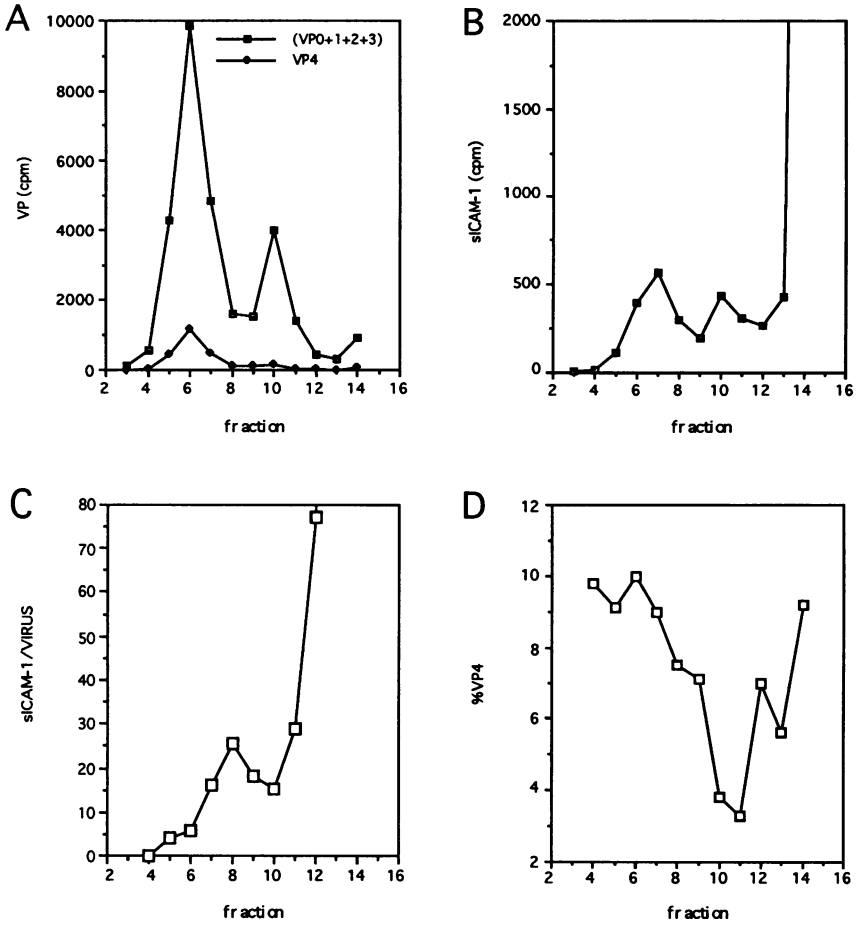

FIG. 6. HRV-3 protein and sICAM-1 quantitation from electrophoresis of sucrose gradient fractions (experiment 1 in Table 1). (A) Radioactivity for VP0 to -3 and VP4 in each fraction; (B) radioactivity for sICAM-1; (C) stoichiometry of sICAM-1 in molecules per particle; (D) percentage of VP4 with respect to VP0 to -3 for each fraction.

results have been obtained. Low pH requirements have been shown to be important for poliovirus and minor-group rhinoviruses in vivo (12, 13, 18). However, a mechanism of poliovirus uncoating in vivo independent of a low pH has been proposed (7), and receptor-induced conversion of poliovirus to inactive 135S particles is unaffected between pH 4.5 and 7.4 (4). It is not known whether entry into the cytoplasm of the RNA and infection by the major group of rhinoviruses occurs through an endosomal compartment or through the plasma membrane. Receptor-mediated endocytosis appears not to be important, because ICAM-1 with a truncated cytoplasmic domain or a glycosyl-phosphatidylinositol membrane anchor is as effective as wild-type ICAM-1 for infection (28).

TABLE 1. Bound-sICAM-1 and VP4 contents of rhinovirus-derived particles

Expt (min of incubation)[a]	sICAM-1 (μM)	% Disruption	No. of sICAM-1 molecules/virion		% Decrease in VP4[b]	
			~120S[c]	80S[d]	~120S	80S
1 (20)	1.1	19.0	13.0 (25.5)	22.0	6.4	64.3
2 (45)	1.5	69.0	22.0 (32.0)	6.0	0.0	71.4
3 (30)	0.9	22.3	23.0 (35.0)	36.3	0.0	32.0
4 (30)	1.1	52.9	29.0 (44.0)	14.6	8.0	71.1
5 (30)	1.6	100.0		3.4		74.0

[a] sICAM-1 and HRV-3 were incubated at 37°C in PIPES buffer (pH 7.0)–5% FCS. Experiments 1 and 2, virus stock 1; experiments 3 to 5, virus stock 2.
[b] With respect to the native virus; calculated from the percentage of VP4 in the combined fractions.
[c] Average for fractions 5 to 8, with the maximum in parentheses.
[d] Average for fractions 10 and 11.

One of the most interesting observations in this study is that sICAM-1 could remain substantially associated with the disrupted 80S particles. VP4 also remained partially associated. We demonstrated association between sICAM-1 and the 80S particles for two different rhinovirus serotypes, HRV-3 and HRV-14. Moreover, we demonstrated the association in multiple experiments and by two independent techniques, sedimentation of radiolabeled sICAM-1 with unlabeled rhinovirus and sedimentation followed by SDS-PAGE of sICAM-1 and rhinovirus that were both radiolabeled. Greve and coworkers previously observed disruption of rhinovirus by sICAM-1 but showed that no sICAM-1 or VP4 was bound to disrupted HRV-14 or HRV-16 80S particles (6, 8). While on the surface these results appear discrepant, they are not necessarily contradictory to our own findings, because our findings indicate that sICAM-1 bound to 80S particles represents an intermediate, rather than a final product, in the uncoating process. A trend in which a higher percent overall virus disruption correlated with a smaller number of sICAM-1 molecules bound per

80S particle was noted. Thus, when HRV-3 stock 2 was incubated with increasing concentrations of sICAM-1, disruption was higher and less sICAM-1 was bound per 80S particle. If all 80S particles had the same structure, the law of mass action would predict that more, not less, sICAM-1 would be bound at higher sICAM-1 concentrations. However, we have found that just the reverse is true, suggesting that there may be heterogeneity among 80S particles. Moreover, our results with VP4 show that the 80S particles are heterogeneous, with VP4 contents that vary from 68 to 26%, and suggest an intermediate state(s) in the disruption process. Our interpretation is that the conformational change that results in release of RNA is a concerted one not for the entire virion but for a smaller portion. For the sake of discussion, it can be assumed that this is a capsid pentamer, since it has been proposed that binding of the receptor could affect the pentamer-pentamer contact and facilitate the exit of VP4 and RNA (20). Thus, a conformational change and release of VP4 could be localized to one pentamer, and RNA could exit through a gap in the contact between that pentamer and the remainder of the virion. Our data are consistent with heterogeneity within a given 80S particle preparation, as well as between different preparations, in the number of pentamers that have undergone disruption. A higher overall percentage of 80S particles correlates, within the population of 80S particles, with less VP4, less bound sICAM-1, and (we hypothesize) with a higher percentage of disrupted pentamers. Thus, sICAM-1 associated with 80S particles may be bound to nondisrupted pentamers.

Disruption dependent on sICAM-1 increased exponentially over 30°C, and very little disruption was obtained below this temperature (8). The kinetics of disruption fit well with the Arrhenius equation, which predicts an exponential increase in the kinetic-rate constant with the temperature in kelvins. Using the Arrhenius equation, we determined the activation energy for the disruption of HRV-3 to be about 42 kcal (ca. 176 kJ)/mol of virus. This substantial energy requirement suggests that a significant rearrangement in viral structure is required to attain the transition between the native and disrupted viral states. Binding of sICAM-1 did not lower the activation energy but lowered the temperature at which disruption occurred by 5°C.

Our finding that 80S particles retain binding to sICAM-1 has important implications for the pathway of infection by rhinovirus. Conformational changes in poliovirus, which can be induced by binding to the receptor (9), have been shown to expose hydrophobic portions of the capsid proteins, allowing attachment to liposomes and probably mediating disruption of the membrane of the host cell and enabling entry of the RNA (3). Binding of the soluble receptor to poliovirus results in formation of a 135S particle that retains RNA and VP4 and excludes stain yet is noninfectious (4, 9). This particle is thought to lack bound receptors, although this has not been explicitly tested. The poliovirus 135S particle may be an expanded particle that is at a position in the uncoating pathway that is intermediate between the infectious 120S and noninfectious 80S particles seen here for rhinovirus. Maintenance of virus binding to its receptor during the uncoating process, as observed here for rhinovirus, would be expected to increase the efficiency of viral RNA entry into the cytoplasm. Our suggestion that rhinovirus capsid disruption progresses in steps, e.g., pentamer by pentamer, rather than all at once, requires further experimental support. This hypothesis has important implications, particularly if entry occurs through the plasma membrane rather than in endosomes. In contrast to the situation with the soluble receptor, binding to ICAM-1 on the cell surface may be localized to the region of the capsid adjacent to the cell membrane. Localized disruption of the virion, polarizing the exit of RNA toward the membrane, should enhance the efficiency of infection.

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