Two Distinct Binding Affinities of Poliovirus for Its Cellular Receptor*

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To study the kinetics and equilibrium of poliovirus binding to the poliovirus receptor, we used surface plasmon resonance to examine the interaction of a soluble form of the receptor with poliovirus. Soluble receptor purified from mammalian cells is able to bind poliovirus, neutralize viral infectivity, and induce structural changes in the virus particle. Binding studies revealed that there are two binding sites for the receptor on the poliovirus type 1 capsid, with affinity constants at 20 °C of $K_D = 0.67 \mu M$ and $K_D = 0.11 \mu M$. The relative abundance of the two binding sites varies with temperature. At 20 °C, the $K_D$ site constitutes approximately 46% of the total binding sites on the sensor chip, and its relative abundance decreased with decreasing temperature such that at 5 °C, the relative abundance of the $K_D$ site is only 12% of the total binding sites. Absolute levels of the $K_D$ site remained relatively constant at all temperatures tested. The two binding sites may correspond to docking sites for domain 1 of the receptor on the viral capsid, as predicted by a model of the poliovirus-receptor complex. Alternatively, the binding sites may be a consequence of structural breathing, or could result from receptor-induced conformational changes in the virus.

Recognition of a cell surface receptor is the first step in infection of cells by animal viruses. For some viruses, interaction with a cell receptor serves only to concentrate virus on the cell surface; release of the genome is a consequence of low pH or the action of proteinases (1). For other viruses such as poliovirus, the cell receptor is also an unzipping and initiates conformational changes in the virus that lead to release of the genome. Poliovirus is an attractive system for studying cell entry, because its three-dimensional structure is known (2) and its action of proteinases (1). For other viruses such as poliovirus, the cell receptor is also an unzipping and initiates conformational changes in the virus that lead to release of the genome. Poliovirus is an attractive system for studying cell entry, because its three-dimensional structure is known (2) and its cell receptor has been identified (3).

The poliovirus virion is composed of 60 protomers, each containing a single copy of the four capsid proteins, VP1, VP2, VP3, and VP4, organized with icosahedral symmetry. Distinguishing features of the virion surface include a prominent peak or mesa at the 5-fold axis of symmetry, a deep surface depression, or canyon, surrounding the 5-fold axis, another protrusion at the 3-fold axis, and a hydrocarbon-binding pocket beneath the canyon floor. The results of genetic and structural analyses demonstrate that the canyon is the receptor-binding site (4–7). The receptor for three poliovirus serotypes (Pvr,† also called CD155) is a type I integral membrane protein that contains three extracellular Ig-like domains, a transmembrane spanning region, and a cytoplasmic tail (3). The results of genetic analyses indicate that domain 1 of Pvr contains the binding site for poliovirus (8–12). Specific interactions between poliovirus and a soluble form of Pvr (sPvr) have been identified by cryo-electron microscopy and image reconstruction of the virus-receptor complex (6, 7). This work demonstrates that all contacts of the receptor with poliovirus involve Pvr domain 1.

The interaction of poliovirus with Pvr at temperatures greater than 33 °C results in dramatic structural rearrangements that lead to the production of altered particles (13–15). These particles lack the capsid protein VP4, and the hydrophobic N terminus of VP1 is extruded to the virion surface. Altered particles can be detected experimentally by their sedimentation rate, 135 S, which differs from native virions (160 S). The altered particle may be an intermediate in the viral entry pathway (16–19). Reversible structural changes occur in the capsids of picornaviruses in the absence of receptors. For example, portions of poliovirus and rhinovirus VP1 and VP4 that are internal in the crystal structure can be detected on the surface of the capsid (20, 21). The translocation of internal proteins to the capsid surface has been called structural breathing. The functional significance of breathing remains unclear, although it is likely to play a role in virus binding and entry. Antiviral compounds that block rhinovirus uncoating also block breathing (21). Poliovirus binding to cells at temperatures below 37 °C is blocked by the antiviral compound WIN51711, suggesting that the viral capsid must undergo structural changes to bind to Pvr (22). In support of this hypothesis, it was shown that the drug does not affect the binding of a poliovirus mutant that is believed to more readily undergo structural transitions (22).

The equilibrium but not the kinetics of poliovirus binding to sPvr has been examined (23). Such parameters are important because they describe the interaction of virus with receptor, which enables a better understanding of the reaction and its comparison to other systems. The results of such studies, together with structural and genetic analyses of the virus-recep-

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† The abbreviations used are: Pvr, poliovirus receptor; pfu, plaque-forming units; sPvr, soluble form of Pvr; IRES, internal ribosome entry site; GFP, green fluorescent protein; PBS, phosphate-buffered saline.
tor interaction, provide a complete picture of early events in infection (24–27). To study the kinetics and equilibrium of poliovirus binding to Pvr, we used surface plasmon resonance (28, 29) to examine the interaction of a soluble form of Pvr (sPvr) with poliovirus. sPvr expressed in and purified from mammalian cells is able to bind poliovirus, neutralize viral infectivity, and induce the formation of altered particles. Surface plasmon resonance revealed that there are two binding sites for Pvr on the poliovirus capsid. The relative abundance of the two binding sites varies with temperature. The two binding sites may correspond to docking sites for domain 1 of Pvr on poliovirus predicted by the model of the poliovirus-Pvr complex (6, 7). Alternatively, the binding sites may be a consequence of structural breathing or could result from receptor-induced conformational changes in the virus.

EXPERIMENTAL PROCEDURES

Cells and Viruses—Recombinant sPvr was produced in the 293-T human epithelial kidney cell line. 293-T cells were propagated in Dulbecco's minimal essential medium (Life Technologies, Inc.) containing 10% fetal bovine serum (HyClone), 100 units of penicillin/ml, and 100 µg of streptomycin/ml (Life Technologies, Inc.). HeLa cells were propagated in Dulbecco's minimal essential medium containing 10% bovine calf serum, 100 units of penicillin/ml, and 100 µg of streptomycin/ml. Hybridoma cell line 711C was propagated in HB basal medium plus HB101 supplement (Irvine Scientific). Poliovirus type 1, Mahoney strain, was grown in HeLa cells and purified by differential centrifugation and CsCl density gradient fraction as described (30). The ratio of particles to plaque forming units was determined to be 250:1.

Plasmid Construction—Polymerase chain reaction was used to amplify a portion of PVR cDNA that corresponds to the ectodomain, residues 1–337. DNA encoding 5 histidine residues and a termination codon were added to the 3′-end during amplification. The 5 histidine residues were added after a naturally occurring histidine (His-337) in PVR. The following sense and antisense primers were used: 5′-tggagagaagggaggtggcagtggagtgtattgggagtgacaattgcta-3′ and 5′-ggaggacatcgttaattttgacttgggtgtgtggtgtcactTGAGGCAGGGCCTCC-3′. Codons for the additional histidine residues are shown in bold. The Pvr sequence is in capital letters. The amplified DNA product was inserted into the first cistron position of the bacterial vector pCMV/IRES/GFP, resulting in p3DPVR/IRES/GFP/MPS. Expression of this DNA in mammalian cells should produce a bicistronic mRNA in which the first cistron encodes sPvr, followed by the encephalomyocarditis internal ribosome entry site (IRES) and the coding region for GFP in the second cistron. In a cell line stably expressing cells with relative fluorescence intensity greater than 95% of secretion of sPvr, as determined by Western blot analysis, was approximately 200 pfu of poliovirus were incubated in 10 mM glycine, pH 3, or PBS, for 5 min, followed by plaque assay. Plaque assays were carried out essentially as described (32).

Binding of sPvr to Poliovirus—Using an Optical Biosensor—Surface plasmon resonance experiments were performed on a BIAcore X and BIAcore 3000 optical biosensor (BIAcore AB) at specified temperatures. Approximately 1,200 response units of purified poliovirus were coupled to flow cell 2 (Fc2) of a CM5 sensor chip via primary amines according to the manufacturer’s specifications with the following modifications. After the activation step, purified poliovirus in PBS was diluted 1:3 with 10 mM sodium acetate, pH 5.5, and injected at 2 µl/min until desired response units were coupled to the flow cell. The running buffer for the experiments was PBS containing 0.005% Tween 20 (PBS-T, pH 7.0). For kinetic analysis of the sPvr-poliovirus interaction, the flow path was set to include both flow cells; the flow rate was 50 µl/min, and the data collection rate was set to high. Poliovirus was allowed to bind for a 2-min interval with a wash delay set for an additional 3 min to allow for smooth dissociation. Settings for equilibrium analyses were the same as for kinetics, except that the flow rate was set to 2 µl/min. Regeneration of the virus (removal of bound sPvr) was done by brief pulses of 10 mM glycine at pH 3.0 with or without 300 mM NaCl until the response was returned to base line. BIAevaluation software, version 3.0, was used to analyze the surface plasmon resonance data, using global fitting.

RESULTS

Expression and Purification of sPvr in Mammalian Cells—A novel approach was used to express a soluble form of the poliovirus receptor at high levels in mammalian cells for biochemical and biophysical studies. A plasmid was used that leads to the production of a bicistronic mRNA upon expression in mammalian cells. The coding region of the Pvr ectodomain (with a 6-histidine tag at the C terminus, Fig. 1) was placed in the first cistron position, followed by an IRES, and then the coding region for GFP in the second cistron. In a cell line stably

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expressing the bicistronic mRNA, the intensity of GFP fluorescence is an approximate indicator of the expression of the protein in the first cistron.\(^2\) Fluorescence-activated cell sorter analysis was then used to isolate a clonal cell line that contains a fluorescence intensity greater than 99.75% of the GFP-positive population. Enriched supernatant from the stable cell line used contained approximately 7 mg/liter sPvr. sPvr was purified from cultured supernatant using a two-step procedure. The level of purification was determined by assaying the capacity of sPvr to neutralize infectious poliovirus (34). In the first step, nickel affinity chromatography achieved 160-fold purification over the cultured supernatant (Table I). In the second step, Q-Sepharose purification ion exchange chromatography achieved 2.3-fold purification over the previous step. At this stage of purification, sPvr was the only visible band on a Coomassie Blue-stained, SDS-polyacrylamide gel (Fig. 1). Edman degradation revealed that the N terminus of purified sPvr begins at Asp-28 of the unprocessed precursor, as previously reported for the membrane-bound form (35). Although the predicted molecular mass of sPvr is 34 kDa, the purified protein migrates as a diffuse band between the 61- and 85-kDa molecular mass markers (Fig. 1), suggesting that the protein is heavily glycosylated. After treatment of sPvr with N-glycosidase F, which cleaves asparagine-linked glycan chains on glycoproteins, the polypeptide migrates at 34 kDa, the predicted size of the non-glycosylated protein (data not shown). A similar protein produced in insect cells migrated at 51 kDa, probably due to less extensive glycosylation in that cell type (23).

**Virus Neutralization and Alteration Activity of sPvr**—We carried out several assays to determine whether purified sPvr is biologically active. Plaque reduction assays were used to determine the efficiency of neutralization of poliovirus by sPvr. Viral infectivity was reduced by 50% at 30 nm sPvr (Fig. 2). In contrast, the 50% inhibitory dose for infectivity (IC\(_{50}\)) of sICAM-1 for rhinovirus type 3 was 10-fold higher than sPvr, 300 nm (36). One possible explanation for this difference is that the affinity of poliovirus type 1 for its soluble receptor is greater than that of rhinovirus 3 for sICAM-1 (see below).

We also determined if sPvr is capable of inducing structural changes in poliovirus. This question was addressed by incubating sPvr with poliovirus at 37 °C and assaying the products by sucrose gradient centrifugation. At the concentration of sPvr used, 1.8 \(\times\) 10\(^{−8}\) M, conversion of native virus (160 S) to 135 S altered particles and 80 S empty capsids was nearly complete within 15 min (Fig. 3). These results indicate that sPvr produced in mammalian cells can efficiently bind to poliovirus and induce the structural changes associated with cell entry.

**Conditions and Specificity of Surface Plasmon Resonance**—Surface plasmon resonance allows determination of quantitative affinities (K\(_D\)), association (k\(_a\)), and dissociation (k\(_d\)) rates for the formation and dissociation of the virus-receptor complex (37–40). To examine the kinetics of binding of sPvr to poliovirus by surface plasmon resonance, purified poliovirus was coupled to the sensor chip surface, and sPvr was injected over the chip surface. An example of raw sensogram data is shown in Fig. 4. In this experiment, flow cell 2 contained immobilized poliovirus, and flow cell 1 was activated and blocked without virus. sPvr was injected, and its association with virus was followed for 2 min. At 120 s, sPvr was replaced with buffer, and the dissociation of complex was followed for 3 min. The response on the y axis is measured in response units. The sensogram reveals a change in the bulk refractive index, but there was no significant background response when 1.3 \(\mu\)M sPvr was injected over the mock-coupled control surface. In the surface plasmon resonance experiments that followed, data from flow cell 2 were subtracted from the data from flow cell 1 to correct for changes in bulk refractive index. These results demonstrate binding of sPvr to poliovirus immobilized on the chip surface.

The sensor chip surface was regenerated by treatment with low pH, to disrupt the virus-receptor interaction. Poliovirus remaining on the chip surface should survive these conditions, because its natural route of infection is through the acidic environment of the stomach. Two experiments were done to ensure that the sensor chips could be reused. First, unbound poliovirus was incubated in regeneration buffer (glycine buffer, pH 3) for 5 min at room temperature, and then infectivity was determined by plaque assay. As expected, this treatment did not reduce poliovirus infectivity, suggesting that conditions used for regeneration of the sensor chip do not disrupt virus structure (Fig. 5). Second, repeated use and regeneration of sensor chips containing bound poliovirus did not affect sensograms and response levels (data not shown).

To determine the specificity of the poliovirus-sPvr interaction, a blocking experiment was performed using a monoclonal antibody, 711C, directed against the first domain of Pvr and which prevents poliovirus attachment to cells (11). Two concentrations of monoclonal antibody 711C were preincubated with sPvr for 1 h at 4 °C prior to injection onto the sensor chip containing bound poliovirus. Preincubation with monoclonal antibody 711C inhibited formation of the virus-receptor complex (Fig. 6). A control monoclonal antibody DL11, directed against herpes simplex virus glycoprotein D, did not inhibit the formation of the poliovirus-sPvr complex (data not shown). These results indicate that the sPvr-poliovirus interaction under study resembles the interaction during infection of cells, since it is mediated by domain 1 of sPvr.

**Kinetic and Equilibrium Affinity Analysis**—Determination of kinetic binding parameters for the sPvr-poliovirus interaction was done at 20 °C using separate injections of 2.5-fold serial dilutions of sPvr onto the sensor chip containing bound poliovirus. The sensograms of the sPvr-poliovirus interaction were imposed upon different model curves generated by global fitting analysis (Fig. 7). The data fit best with the parallel reactions (2 sites) model, A + B1 ⇌ AB1, A + B2 ⇌ AB2. The X\(^2\) values generated using this model for interaction at 20 °C were below 1.5, indicating an excellent fit. On the other hand, the X\(^2\) value for a one-site binding model was 29, demonstrating a poor fit to that model. The two affinity constants calculated from the surface plasmon resonance data are 0.67 \(\mu\)M (K\(_{D1}\)) and 0.11 \(\mu\)M (K\(_{D2}\)) (Table II). The calculated association rate constants are 3.6 \(\times\) 10\(^{3}\) M\(^{−1}\) s\(^{−1}\) (k\(_{a1}\)) and 3.2 \(\times\) 10\(^{4}\) M\(^{−1}\) s\(^{−1}\) (k\(_{a2}\)); the dissociation rate constants are 2.4 \(\times\) 10\(^{−3}\) s\(^{−1}\) (k\(_{d1}\))

\(^2\) T. Livelli, personal communication.
and 3.3 \times 10^{-3} \text{ s}^{-1} (k_{d2}) (Table II). Binding rates were unaffected by changes in flow rate, demonstrating that the poliovirus-sPvr interaction is not limited by mass transport (data not shown) (41). The kinetics and affinity analysis of the rhinovirus-sICAM interaction using the biosensor, as well as affinity analysis in solution, was also shown to be biphasic (38). In that study, the linear transformation method was used to analyze biosensor data on the rhinovirus-sICAM interaction. This method, when applied to our data on the poliovirus-sPvr interaction, also yields biphasic plots indicative of two binding sites (data not shown).

Binding of sPvr to the sensor chip was repeated under equilibrium conditions to confirm the existence of two classes of binding sites, and the affinity constants were determined by Scatchard analysis (42). The contact time was varied from 50 min for the lowest concentration to 10 min for the highest concentration of sPvr (Fig. 8A). The Scatchard plot of the equilibrium data is curved, indicating that there are two classes of sPvr-binding sites on poliovirus at 20 °C, with binding affinities of 1.1 \mu M (K_{d1}) and 0.16 \mu M (K_{d2}) (Fig. 8B) (43). These values are similar to those obtained by kinetic analysis (Table II).

To determine the effect of temperature on the poliovirus-receptor interaction, the kinetics experiments were repeated at 5, 10, 15, and 20 °C. Higher temperatures, at which receptor-induced virus disruption occurs, were not studied because it would be difficult to interpret the biosensor data (44). With increasing temperature, the value for K_{d1} decreased, indicating a rise in affinity (Table III). Binding of sPvr at these sites on poliovirus is therefore endothermic. The value for K_{d2} did not exhibit a general increase or decrease with temperature, and therefore the thermodynamic nature of this site could not be determined.

The relative abundance of the K_{d1} and K_{d2} sites at different temperatures was calculated from the kinetics data using glo-
has not been described previously. The binding affinity of poliovirus for the surface of HeLa cells was previously determined to be approximately $10^{-10} \text{ M}$ at $4 {^\circ}\text{C}$ (45, 46). We find that the binding affinity of the $K_{D1}$ site, the predominant binding site at this temperature, is 4 orders of magnitude lower. The difference may be explained by the fact that the binding affinities calculated in the present study represent the intrinsic affinity of poliovirus for a single receptor molecule. In contrast, receptor molecules may cluster on the cell surface, increasing the apparent affinity, or avidity, of the virus-receptor interaction. Such clustering does not occur in solution (36). In another study, a single binding affinity of poliovirus for a soluble form of Pvr produced in insect cells was determined to be $4.5 \times 10^{-8} \text{ M}$ at $4 {^\circ}\text{C}$ (23). In those studies, binding assays were conducted in plastic microtiter plates. Although the affinity of this site is similar to that of the $K_{D2}$ site, it is not clear why the lower affinity site was not detected. One possibility is that concen-

**DISCUSSION**

To measure kinetic constants of the poliovirus-receptor interaction, we expressed and purified from mammalian cells a soluble form of the poliovirus receptor. Surface plasmon resonance was used to study binding of poliovirus with sPvr. The affinities determined by biosensor are within 1 order of magnitude of the IC$_{50}$ of sPvr determined by plaque assay, suggesting that the values determined by BIAcore could be the functional affinities for sPvr. The results indicate that the interaction between poliovirus and sPvr is biphasic. Two classes of binding site for sPvr on poliovirus were detected, called the $K_{D1}$ site and the $K_{D2}$ site. At $5 {^\circ}\text{C}$, approximately 90% of the binding sites were $K_{D1}$ sites, with a binding affinity of 1.56 $\text{mM}$. The fraction of $K_{D2}$ sites, with a binding affinity of 0.11 $\text{mM}$, increases with temperature and constitutes 50% of the sites at $20 {^\circ}\text{C}$. A biphasic binding model for poliovirus and Pvr analysis software, assuming a parallel reactions model. At $20 {^\circ}\text{C}$, the $K_{D2}$ site constituted approximately 46% of the total binding sites on the sensor chip (Table III, %R$_{\text{max}}$). The relative abundance of the $K_{D2}$ site decreased with decreasing temperature. At $5 {^\circ}\text{C}$, the relative abundance of the $K_{D2}$ site is only 12% of the total binding sites. Absolute levels of the $K_{D1}$ site remained relatively constant at all temperatures tested.
trations of sPvr were not sufficiently high to detect the lower affinity site. In addition, proteins produced in insect cells and in mammalian cells have different patterns of glycosylation, which might contribute to the different results. An N-linked glycosylation site within Pvr domain 1 is known to influence its interactions with poliovirus (12) and may contact the receptor binding site on the viral capsid (6). A side-by-side comparison must be done to resolve this issue.

The finding of two classes of receptor-binding sites on a virus has also been reported for rhinovirus type 3 and a soluble form of its cellular receptor, ICAM-1 (38, 47). Although the rhinovirus-sICAM and poliovirus-sPvr interactions are biphasic, there are significant differences in the affinity and kinetic constants. The association rates $k_{a1}$ and $k_{a2}$ are 25- and 13-fold higher for the poliovirus-sPvr interaction than for the rhinovirus-sICAM interaction at 20 °C. The greater association rate of poliovirus-sPvr might be due, in part, to differences in the extent of contact between virus and receptor. Three-dimensional models of virus-receptor complexes produced from cryo-electron microscopy and image reconstruction reveal that the footprint of Pvr on poliovirus is significantly larger than that of ICAM-1 on rhinovirus (6, 7, 48). The extra surface area on poliovirus includes the knob of VP3 and the C terminus of VP1 from the 5-fold related promoter in the southeast corner of the road map describing the contact of Pvr on poliovirus (6). In contrast, although there are two dissociation rate constants for poliovirus-sPvr, only one has been reported for the rhinovirus-sICAM interaction (38, 47). The dissociation rates for the poliovirus-sPvr interaction are 1.5- and 2.0-fold faster than for the rhinovirus-sICAM interaction, indicating greater instability of TABLE II

**Kinetic and Affinity Analysis of Poliovirus Binding to Pvr**

| Kinetic constants were measured as described in Fig. 7 for binding of sPvr to poliovirus type 1. |
|---|---|---|---|---|---|
| $k_{a1}$ | $k_{a2}$ | $k_d1 \times 10^{-3}$ | $k_d2 \times 10^{-3}$ | $K_{D1}$ | $K_{D2}$ |
| $3,600 \pm 660^{a}$ | $32,000 \pm 2,600$ | $2.4 \pm 0.9$ | $3.3 \pm 0.4$ | $0.67 \pm 0.28$ | $0.11 \pm 0.02$ |

$^{a} K_{D} = k_d/k_a$.

$^{b}$ Mean of three experiments ± S.D.

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**FIG. 8.** Equilibrium binding sensorgrams and Scatchard analysis of the binding of sPvr to immobilized poliovirus. A, binding of sPvr to immobilized poliovirus was monitored for 10 min for the injections of 13.5 (red line), 9 (blue line), 6 (green line), 4 (magenta line), and 2.67 (turquoise line) μM concentrations; 20 min for injections of 1.78 (gold line), 1.18 (black line), 0.79 (yellow line), 0.53 (pale blue line), and 0.35 (pink line) μM concentrations; and 50 min for injection of the 0.23 μM (salmon line) concentration. Arrows indicate the time points used for the Scatchard analysis. B, Scatchard analysis. C (ordinate legend) is the concentration of sPvr flowed across the sensor chip surface at 20 °C. The negative slope of each line is equal to each association constant; the reciprocals are the $K_{D}$ values. The $R^2$ values for the linear fit of the data were 0.95 and 0.91 for $K_{D1}$ and $K_{D2}$, respectively.
The affinity constants for the poliovirus-sPvr interaction are 19- and 6-fold greater than those reported for the rhinovirus-sICAM-1 complex (38). Consistent with these differences is the fact that the IC₅₀ of sICAM-1 for rhinovirus 3 is 10-fold higher than that of poliovirus (36). However, other factors might play a role, including the number of receptors per virus particle that are required to neutralize infectivity.

The effect of temperature on the interaction of poliovirus with sPvr was studied. Binding at the lower affinity site, KD₂, is endothermic (e.g. heat is absorbed by the complex), similar to both sites on rhinovirus (38, 47). As suggested previously, heat absorbed during the interaction of virus with receptor might help to lower the energy barrier required for uncoating of the virus particle (38).

In contrast to the observations with poliovirus and rhinovirus, a single class of binding site (KD₁ = 3.0 × 10⁻⁶ m at 20 °C) was found on echovirus 11 for a soluble form of its receptor, CD55 (39). The affinity of this interaction is at least 4 times lower than either of the binding sites on poliovirus for sPvr. Like most protein-protein interactions, the affinity of echovirus 11 for CD55 increases with decreased temperature, indicating that binding is exothermic. The association rate for the interaction between echovirus 11 and CD55 is faster than that of poliovirus-sPvr (39- and 4.4-fold) and rhinovirus-sICAM-1 (38). One explanation for these findings is that the contact between echovirus 11 and CD55 is more extensive than that of the other two virus-receptor complexes. In addition, the binding site for CD55 on echovirus 11 might be more accessible than those of Pvr and ICAM-1, which are located in a depression on the capsid (6, 7). The dissociation rate for the echovirus-CD55 interaction is at least 97 times faster than that of either poliovirus-sPvr or rhinovirus-sICAM-1 (38). These findings are consistent with a more accessible binding site for CD55 on echovirus 11, compared with the receptor-binding sites on poliovirus and rhinovirus (38, 39). In addition, it is possible that the atomic interactions between CD55 and echovirus 11 are weaker than between the other two viruses and their receptors. The faster dissociation rate of the echovirus-CD55 complex may be related to the finding that the interaction does not lead to structural changes of the virus particle (49), as occurs with poliovirus and rhinovirus. The lower dissociation rates for the poliovirus- and rhinovirus-receptor complexes may in part reflect the time required for structural changes to occur. Elucidation of the high resolution crystal structures of all three virus-receptor complexes should provide explanations for the differences in kinetic parameters.

Why do poliovirus and rhinovirus have two classes of receptor-binding sites? One possibility is suggested by a three-dimensional model of the poliovirus-sPvr complex (see Fig. 3 in Ref. 6). In this model, domain 1 of sPvr contacts two major sites on the virus surface, one in a cleft on the “south rim” of the canyon and a second on the side of the mesa on the “north rim.” Whether these two contact sites correspond to the two classes of binding sites can be tested by carrying out kinetic and equilibrium binding studies on viruses with amino acid changes in these areas (4). Since all contacts of Pvr with the virus involve domain 1 (Fig. 9), the finding of two classes of binding sites cannot be explained by the involvement of Pvr domains 2 and 3. Two classes of binding sites might also be a consequence of the structural flexibility exhibited by both viruses. Normally internal parts of the poliovirus and rhinovirus capsid proteins have been shown to be transiently displayed on the virion surface, a process called breathing (20, 21). Interaction of poliovirus and rhinovirus with their cellular receptors leads to irreversible and more extensive structural changes (19, 34, 50, 51). Antiviral drugs, such as WIN compounds, which replace the lipid-like molecule in the hydrophobic pocket, are believed to block uncoating of the capsid by rendering it structurally rigid (52). Binding of poliovirus to its cellular receptor may cause release of the lipid-like molecule the hydrophobic pocket, allowing the capsid to undergo structural transitions necessary for binding and entry (6). Such structural plasticity might explain the presence of two different classes of binding sites on the virion. At lower temperatures, the higher affinity binding site is less abundant compared with the lower affinity site. At higher temperatures, the relative abundance of the higher affinity site is increased compared with the lower affinity site. One explanation for these observations is that increased breathing of the virus at higher temperatures results in the exposure of the higher affinity site. In addition, the interaction between receptor and virus may induce a conformational change in the capsid that results in exposure of the higher affinity binding site. In contrast to the findings with poliovirus and rhinovirus, binding of echovirus 11 with CD55 can be described by a simple 1:1 binding model. Such behavior, which would be expected for the interaction of two preformed binding sites, is consistent with the fact that the echovirus-CD55 interaction does not result in detectable structural changes in the capsid (49).

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