Kinetic Models for Receptor-Catalyzed Conversion of Coxsackievirus B3 to A-Particles

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ABSTRACT

The immunoglobulin superfamily protein receptors for poliovirus, human rhinovirus, and coxsackievirus B (CVB) serve to bind the viruses to target cells and to facilitate the release of the virus genome by catalyzing the transition from the mature infectious virus to the A-particle uncoating intermediate. Receptor binding sites characterized by two equilibrium dissociation constants have been identified. The site with higher affinity is best observed at warmer temperatures and appears to correlate with the reversible conformational state in which the capsid is permeable to small molecules and peptides that are buried in the crystal structures are exposed. Measurements of CVB conversion to inactive particles over time in the presence of varied concentrations of soluble coxsackievirus and adenovirus receptor showed that the observed first-order rate constant varies with receptor concentration. The dose-response data, previously modeled as the sum of first-order reactions, have been used to evaluate models for the receptor-catalyzed conversion of CVB that include the high- and low-affinity binding sites associated with capsid breathing. Allosteric models wherein receptor binding shifts the equilibrium toward the open capsid conformation, in which the high-affinity binding site is available, best fit the data.

IMPORTANCE

This paper compares models that relate the structural, mechanistic, and kinetic details of receptor-virus interactions known from previous work with human enteroviruses. New models are derived using recent results from receptor-catalyzed conversion of coxsackievirus B3 to non-infectious A-particles. Of those considered, the acceptable models include the capsid breathing cycle and two conformation-dependent receptor binding sites. The results indicate that the receptor enhancement of virus conversion to A-particles involves allostery through conformation selection.

Among the enteroviruses (Picornaviridae) that use immunoglobulin superfamily proteins as cell surface receptors, the events that initiate when a virus binds a receptor and that culminate with genome uncoating have been elucidated in considerable though incomplete detail, primarily from studies of poliovirus (PV; Enterovirus C), human rhinoviruses (HRVs; Rhinovirus A), and group B coxsackieviruses (Enterovirus B). These viruses all haveicosahedral protein capsids that enclose the RNA genome and, in the infectious uncoating process, they transition through several metastable states, intermediates with sufficient stability to be isolated and characterized, from the mature virion to the empty capsid. These metastable states include the infectious virus with mature capsid comprised of proteins Vp1 to Vp4 and an enclosed RNA genome, for which atomic-level structures have been determined. Subsequent to the interaction of mature poliovirus, coxsackievirus B (CVB), and receptor-sensitive serotypes of human rhinovirus with receptors at physiological temperatures (or when heated), the A-particle is generated. The A-particle retains the intact genome inside an expanded capsid on which previously buried parts of Vp1 are exposed and from which Vp4 has been lost. Atomic-level structures for the A-particle have also been determined. The genome contained in the A-particle remains capable of generating virus progeny if introduced into a host, but in most cases, the inability of the A-particle to bind the receptor renders the A-particle relatively noninfectious via the receptor-mediated route. In the final capsid metastable state, the capsid, like the A-particle, is expanded (about 4% relative to the mature virus), the amino-terminal epitope of Vp1 remains exposed, and the RNA genome has been discharged. Virus in these metastable states can be isolated based on their buoyant densities. This is a relatively mature area of investigation, and these details are covered in chapters 4 to 6 of The Picornaviruses (1–3) and reviewed by Hogle (4).

In addition to the metastable states, a transient, reversible state has been identified in which normally buried parts of Vp1 and Vp4 are exposed (5–7) and the capsid is permeable to small molecules (8). This transient state is not observed at temperatures much below physiological temperatures (37°C; 34 to 35°C for rhinovirus) and is described in the context of reversible changes in capsid structure called breathing. In this view, the breathing capsid cycles between the closed state (an impermeable capsid with the Vp1 N-terminal region and Vp4 buried) and the open state (a permeable capsid with the Vp1 N-terminal region and parts of Vp4 exposed). Comparison of HRV3–intracellular adhesion molecule-1 (ICAM-1) complexes obtained with and without brief incubation at 37°C revealed an expansion of the capsid with potential new receptor-capsid contacts in response to the higher temperature, consistent with an expanded open state and a conformationally altered receptor binding site at the canyon (9). The expanded state of the poliovirus capsid that occurs at 37°C has also...
been observed after trapping it with antibody to the amino-terminal region of Vp1, which is reversibly exposed in the open state (10). Breathing likely corresponds to low-frequency modes of capsid dynamics and, in particular, modes that are altered by occupancy of the hydrophobic cavity in Vp1 (6, 11, 12). The open and closed conformations coexist in a temperature-dependent equilibrium. Conceptually, the open state lies between the closed state and the A-particle, since breathing is a reversible cycle between conformations while conversion to A-particles is not reversible, and antivirals that block breathing also stabilize the capsid against conversion to the A-particle (6, 13).

The poliovirus receptor (PVR), coxsackievirus and adenovirus receptor (CAR), and ICAM-1 function to tether their respective viruses to targeted host cells, and they participate in the conversion of the mature virus to the A-particle. The details of the transition between the mature virus and A-particle and receptor function appear to be more complex than may have been anticipated. The poliovirus receptor lowers the activation energy required for the poliovirus transition to the A-particle, consistent with transition state theory catalytic activity (14). The receptors (PVR, CAR, and ICAM-1) bind their respective viruses (poliovirus [PV], coxsackievirus B [CVB], and human rhinovirus [HRV], respectively) at structural recesses (called the canyon) around the 5-fold axes of symmetry, but two distinct binding constants have been observed. ICAM-1 was reported to bind HRV3 with equilibrium dissociation constants ($K_{d}$ values) of 600 to 800 nM and 6 to 12 μM at 15 to 20°C; binding was endothermic (15). The suggestion that binding to the low-affinity sites, with lower enthalpy, would precede binding to the high-affinity sites, with greater enthalpy, seems intuitively relevant to the temperature-dependent breathing cycle (15). Subsequent work reported equilibrium dissociation constants as approximately 300 nM and 2 μM for HRV3–ICAM-1 (190 nM for the high-affinity site at 37°C by extrapolation) and 240 nM and 4 μM for HRV16–ICAM-1 at 20°C (16). While the latter study reported finding only a single 200 nM $K_{d}$ at 20°C (calculated to be 80 nM at 37°C) for PV interaction with PVR, other work (17) found two equilibrium dissociation constants for PV-PVR, with values of 110 to 160 nM and 670 to 1100 nM at 20°C. The high-affinity site abundance increased as temperature was raised, leading the authors to speculate that the high-affinity binding site becomes available due to capsid breathing (17) and supports the suggestion that temperature-dependent structural changes enhance PV binding of the PVR (18). The $K_{d}$ of the low-affinity site decreased from 1,560 nM at 5°C to 670 nM at 20°C. The single equilibrium dissociation constant reported for the CVB3 (strain CG)-CAR interaction was 245 nM (the temperature was not specified (19)).

Binding constants have not been directly ascertained at physiological temperatures because the viruses usually convert to A-particles. Nevertheless, the observations of temperature-dependent reversible conformational changes (breathing) and temperature-dependent binding sites have led investigators to propose that the higher-affinity binding must involve a conformation-dependent site that is available only on the open state of the capsid, presumably at or very near the low-affinity site at the canyon (3, 17). Because receptor binding to the high-affinity sites at physiological temperatures catalyzes A-particle formation, the structures for most virus-receptor complexes have been solved at low temperatures and must correspond to the low-affinity binding mode.

**METHODS, RESULTS, AND DISCUSSION**

The availability of a complete dose-response curve for the transition of soluble CAR-catalyzed coxsackievirus B3 (CVB3) to noninfectious A-particles at 37°C (20) enables evaluation of kinetic models for comparison with existing mechanistic models. As previously reported, these enteroviruses transition to noninfectious A-particles (decay) with first-order kinetics in the absence or presence of a receptor, but the presence of a receptor increases the apparent rate constant, $k_{app}$ (14, 20).

From previous analysis (20) of the $k_{app}$ versus soluble-CAR (sCAR) data and the reaction model in equation 1,

$$V + R \rightleftharpoons RV \rightarrow A + R$$

where $V$, $R$, and $A$ represent the concentrations of virus ($V$), receptor ($R$), and A-particle ($A$), respectively, $k_{app}$ was described equally well by

$$k_{app} = k_{1} + k_{2} \cdot \frac{R}{K + R}$$

or

$$k_{app} = k_{1} \cdot \frac{K}{K + R} + k_{2} \cdot \frac{R}{K + R}$$

Best-fit solutions for equations 2 and 3 (Fig. 1A); values for $k_{2}$ and $K$ that best fit equation 3 to the data are presented in Table 1, row A. These values differ slightly from those of the previous analysis (20) due to the use of replicate values rather than averages and omission of a low data point at a 500 nM receptor concentration (Fig. 1A). Examination of residuals (Fig. 1A) suggests that data deviations from the models represented by equations 2 and 3 are systematic. The hypothesis that the deviations are random was rejected (runs test, $P < 0.05$), indicating that the models do not fully describe the data. The simple cooperativity model mentioned previously (20) is plotted in Fig. 1B. The residuals for this model show more random distribution around zero, and the hypothesis that they are randomly distributed is not rejected. So, the cooperative model (Table 1, row B; Fig. 1B) provides an acceptable description of the data, but it is an empirical fit that ignores the conformational intermediates that result from capsid breathing.

At this point, it is useful to recall that $K_{r}/(K_{r} + R)$ is the proportion of total binding sites that are vacant and that $R/(K_{r} + R)$ is the proportion of total receptor binding sites that are occupied and to consider how the proportion of total sites bound relates to the proportion (or number) of sites bound per capsid. The data in Fig. 1 were obtained with $R$ much greater than $V$, so $R_{free}$ is approximated by total $R$. There are 60 receptor binding sites on each virus capsid, and this adds considerable complexity to the problem if the sites interact (i.e., if binding at one site alters the probability of binding at a second site on the same capsid). If the sites are independent, the number 60 appears to be sufficiently large that the proportion of sites occupied among all sites approximates the proportion of sites bound on average per capsid. For example,
if 50% of all sites are bound \[ R/(K_d + R) = 0.5 \], then the percentages of capsids with more or fewer sites bound are normally distributed around the average value of 30 sites per capsid (Fig. 2). The distributions of capsids with different numbers of sites occupied become distorted as average site occupancy approaches the extreme limits (0, 60), but the average is still represented by the proportion of total independent sites occupied times 60.

With introduction of capsid breathing, the equilibrium between the closed and open states (represented by \( V \) and \( U \), respectively) of the mature infectious virus must also be considered. In the absence of a receptor, \( V \leftrightarrow U \rightarrow A \) (4)

where \( K_{eq} \) is the conformational equilibrium constant, and \( k \) is the first-order rate constant for transition of \( U \) to \( A \). Conditions that increase the concentration of open-state capsid will increase the rate of virus decay, because \( dA/dt \) is equal to \( k \) times \( U \).

In equation 4, the open state is an intermediate between the closed state of the mature virus and the A-particle. Now \( k_1 \) in equations 1 to 3 includes both \( k \) and \( K_{eq} \)

\[
k_1 = k \cdot \frac{K_{eq}}{K_{eq} + 1}
\]

(5)

When a receptor is added (Fig. 3), the virus states increase from the 3 in equation 4 to over 100 (including all \( VR_i \) and \( UR_j \), where \( i \) and \( j \) equal 0 to 60) or to relatively unlimited states if the possible geometric combinations of bound and empty sites are not equivalent. At any given value for the receptor \( R \), there will be a population of \( VR_i \) centered on the average occupancy \( 60 \cdot [R/(R + K_d)] \), and a population of \( UR_j \) also centered on the average occupancy \( 60 \cdot [R/(R + K_d')] \), that will be considered in the analyses. The equilibrium constant for the open and closed states is given by \( k_1/K_{eq} \), when \( R \) is zero and by \( k_1'/K_{eq}' \), when receptor binding sites are occupied. The left side of the scheme (Fig. 3), when \( R \) equals 0, has been solved per equation 5, with \( k_1 \) equal to 0.1/h.

**FIG 1** The apparent first-order rate constant for coxsackievirus conversion to A-particles varies with receptor concentration (20). (A to E) Nonlinear least-squares fits of models (Table 1) to the data, with residuals. (A) Rectangular hyperbola (equation 3); (B) equation 3 with cooperativity; (C) simple breathing with \( k \) and \( k' \) different; (D) wedge model (equation 10); (E) \( R \) affecting both \( k_1' \) and \( k'' \), producing the \( k_1'K_{eq}' \) model described by equation 12; (F) MWC model (equation 13). Residuals are plotted in the lower panels. The \( k_{app} \)-versus-sCAR data set was previously published (20) as the average of values from the duplicate assays shown here. (A) A single point (open circle) has been omitted from the original data set. In all experiments, \( R \) is \( \gg V \), so \( R_{free} = R_{total} \). The hypothesis that residuals are randomly distributed is rejected at a \( P \) of 0.05 for panels A, C, and D (runs test).
The right side of the scheme, when $R$ approaches saturating concentrations, is solved as

$$k_2 = \frac{k' \cdot K_{eq}'}{K_{eq} + 1} \quad (6)$$

One estimate of $k_2$ (1.85/h) is available from fitting the cooperativity model (Table 1, row B) to the data (Fig. 1B).

Appreciating that the rate of virus decay when $R$ is zero is given by $k \cdot U$, and by $k' \cdot UR$, when receptor binding sites are saturated, three possible mechanisms for the kinetic effects (change in $k_{app}$) of receptor binding are considered. First, there may be different rates ($k_i$) that apply to each possible number of sites or combination of occupied sites. Second, receptor binding may shift the open/closed equilibrium (i.e., there are different $K_{eq}$ values that apply to each possible number of occupied sites or combination of occupied sites) in favor of the open conformation (U and UR) in proportion to sites occupied, with a single value of $k$, so the rate of decay $[k \cdot (U + UR)]$ increases due to increasing $U + UR$. Third, the receptor may affect both $k$ and $U + UR$.

The probability that a virus will be in the open state (with a high-affinity site) increases with increasing temperature (17), conversion of a virus to A-particles is accelerated by heat (13, 21), compounds that stabilize the capsid (i.e., inhibit conversion to A-particles) also diminish breathing (6, 18), and established models for allostery argue that increasing $R$ will shift virus toward $UR$ if $K_d > K_{eq}$ (22). Assuming that there is a single $k$ that applies to all $U$ and $UR_i$ values and that $k$ is rate limiting simplifies the

| Table 1: Kinetic models and best-fit solutions$^a$ |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Model          | Equation        | Parameters Fit (Constraints) | $r$  | $SSR$ | $MSC$ |
| Hyperbola      | $k_{app} = k_1 \cdot \frac{R}{(K + R)} + k_2 \cdot \frac{R}{(K + R)}$ | $k_1 = 1.98/\text{hr}, K = 55\text{nm}$ ($k_2 = 0.1/\text{hr}$) | 0.9956 | 0.0773 | 4.27 |
| Cooperative    | $k_{app} = k_1 \cdot \frac{R}{(K + R)} + k_2 \cdot \frac{R}{(K + R)}$ | $k_2 = 1.85/\text{hr}, K = 80 \times 10^{12}$ ($k_1 = 0.1/\text{hr}$) | 0.9986 | 0.0209 | 5.45 |
| Breathing      | $k_{app} = \left(\frac{k \cdot K_d + k' \cdot R}{R + K_d} + \frac{K_{eq}}{1 + K_{eq}}\right)$ | $k = 0.18/\text{hr}, k' = 3.51/\text{hr}, K_d = 45\text{nm}$ ($K_{eq} = 0.1/(k - 0.1)$) | 0.9960 | 0.0963 | 3.92 |
| Wedge          | $k_{app} = k \cdot \frac{R + K_d}{R + K_d + K_{eq}}$ | $k = 1.98/\text{hr}, K_d = 5\text{nm}$ ($K_{eq} = 0.1/(k - 0.1)$) | 0.9956 | 0.0773 | 4.27 |
| $k_i/k_{eq}'$  | $k_{app} = k \cdot \frac{K_{eq}'}{K_{eq}} \cdot (R + K_d) \cdot (R + K_d)$ | $k = 1.82/\text{hr}, K_d = 44\text{nm}, K_d = 6\text{nm}$ ($K_{eq} = 0.1/(k - 0.1)$) | 0.9990 | 0.0147 | 5.80 |
| MWC            | $k_{app} = k \cdot \frac{K_{eq}'}{K_{eq}} \cdot (R + K_d + K_{eq}') \cdot (R + K_d)$ | $k = 2.08/\text{hr}, K_d = 41\text{nm}, K_d = 37\text{nm}$ ($K_{eq} = 0.1/(k - 0.1)$) | 0.9986 | 0.0213 | 5.43 |

$^a$ Nonlinear least-squares fitting used the Powell method implemented in PSI-Plot (v.10.5; Poly Software International, Pearl River, NY). $r$ is the correlation coefficient, SSR is the sum of squares of residuals, and MSC is the model selection criterion, all as calculated in PSI-Plot.

$\text{FIG 2}$ Percentages of capsids with 0 to 60 occupied receptor binding sites at different values of $R/K_d$. Assuming independent sites, the occupancy of each site in a population of 10,000 capsids at each concentration of $R$ was assigned as true if a random number (0 to 1) generated for each site was less than or equal to the value of $R/(K_d + R)$.

$\text{FIG 3}$ Capsid breathing and observations of receptor binding sites with different affinities suggest that capsids exist in closed (V) and open (U) states with different dissociation constants for the receptor ($R$). The equilibrium between V and U is influenced by receptor binding.
following analyses and is consistent with the assumption that the receptor catalyzes virus decay to A-particles by altering $K_{eq}$ (stabilizing the open conformation). This rationale is schematically presented in Fig. 4, where subscripts for VR and UR have been omitted since the proportion of total sites occupied can represent the proportion of sites occupied per capsid, as discussed above.

With

$$k_{app} = k \cdot \frac{(U + UR)}{V_{total}}$$

solving for the relative concentrations of each of the species in Fig. 4 that are in equilibrium (V, VR, U, UR) in terms of $V_{total}$ (Fig. 5), with a single value for $k$ ($k = k'$), $k_{app}$ is derived as

$$k_{app} = k \cdot \frac{K_{eq}}{K_{eq} + 1}$$

where $K_{eq}$ is given by $k_f/k_r$ (same as $k'/k''$ here). This model is independent of $K_d$ and $K_{d}/K_{eq}$ and, unless $K_{eq}$ changes with changes in $R$, is not consistent with the observation that $k_{app}$ increases with increasing receptor concentration. [The solution for breathing in which $K_{eq}$ is not affected by $R$, and $k$ is different from $k'$ (Table 1, row C), is equivalent to equation 3 multiplied by a constant equal to $K_{eq}/(K_{eq} + 1)$, fits the data no better than equation 3, and is still independent of $K_d$.]

$K_{eq}$ can vary if receptor binding affects $k_f/k_r$, or both. Xing et al. (9, 16) suggested a wedge model of receptor action wherein the binding of receptor to the open-state high-affinity site of the capsid impedes the return to the closed capsid conformation. Such a mechanism should affect $k'_f$ and can be accommodated in the scheme in Fig. 4 if receptor binding to the open-state capsid diminishes $k'_f$; e.g.,

$$k'_f = k \cdot \frac{K'_d}{R + K'_d}$$

Since $R$ does not affect $k_f$ in this model, $k'_f$ equals $k_f$ and

$$k_{app} = k \cdot \frac{R + K'_d}{R + K'_d + K''_d}$$

The independence of $k_{app}$ from $K_d$ in equation 10 is still unsatisfying. Moreover, the least-squares fit for equation 10 (Fig. 1D; Table 1, row D) is not different from the fits provided by equations 2 and 3 (Fig. 1A; Table 1, row A), and the analysis of residuals indicates that the model does not adequately describe the data. However, the wedge model may provide half the solution if a rational approach that includes a $K_d$ contribution to $k'_f$ can be developed.

When the capsid is in the open state, it will acquire receptor to the high-affinity binding site from solution, and establish the equilibrium defined by $K'_d$. If, however, the receptor is already bound to the low-affinity site of the closed state when the breathing transition occurs, the bound receptor will be near, if not pre-
ciscely at, the high-affinity site, and only the binding transition from weak to tight needs to occur (i.e., the acquisition-from-solution component is bypassed). This situation is depicted in Fig. 6, where the conformational equilibrium is determined by \( k^f/k^o \) and the binding transition is characterized by forward and reverse rate constants, \( k^f \) and \( k^r \). \( k^f \) and \( k^r \) now include rates for both steps. In this scheme, \( k^r \) can be increased relative to \( k^f \) by a receptor bound to the low-affinity site in the closed state. Such an increase can be modeled by

\[
k^r' = k^r \cdot \frac{R + K^d}{K^d}
\]

and with \( k^r' \) from equation 9,

\[
k_{app} = k \cdot \frac{K_{eq}}{K_{eq} + \left(\frac{R + K^d}{K^d} \cdot \frac{K^d'}{R + K^d'}\right)^{\frac{1}{m}}}
\]

Note that when \( R \) is equal to 0, equation 12 is the same as equation 8, but as \( R \) approaches infinity, the value for \( k_{app} \) goes to \( k \). Equation 12 fits the data with \( k^f \) equal to 1.82/h, \( K^d \) equal to 44 nM, \( K^d' \) equal to 6 nM, and calculated \( K_{eq} \) equal to 0.058 (Fig. 2E). The symmetry in equation 12 allows the values for \( K^d \) and \( K^d' \) to be interchangeable, so it is the direct measurements of high- and low-affinity sites cited above that allows assignment of \( K^d \) to be greater than \( K^d' \). Analysis of residuals indicates that the model provides an acceptable description of the data (the hypothesis that the residuals are randomly distributed is not rejected). The model selection criterion (MSC) (23) (Table 1) further indicates that this is the best-fit model among those considered.

Systems with components that exist in multiple conformations, each of which binds a ligand(s) with different affinities (such as the scheme shown in Fig. 3, 4, and 6), are allosteric systems. The equation derived for this scheme with a single \( k \) from the MWC model based on the equilibrium between two conformations (22) has

\[
k_{app} = k \cdot \frac{K_{eq}}{K_{eq} + \left(\frac{R + K^d}{K^d} \cdot \frac{K^d'}{R + K^d'}\right)^{\frac{1}{m}}}
\]

The least-squares fits of equation 13 to the data varied slightly with different initial estimates. The best fit from six attempts gave a k of 2.08/h, a \( K^d \) of 41 nM, and a \( K^d' \) of 37 nM, with a calculated \( K_{eq} \) of 0.05, and describes a curve (Fig. 1F) visually similar to that described by equation 12 (Fig. 1E) and a near match to the simple cooperativity curve (Fig. 1B). Again, analysis of the residuals indicates that the model provides an acceptable description of the data. When \( R \) is equal to 0 in equation 13, \( k_{app} \) is again the same as in equation 8, but as \( R \) approaches infinity,

\[
k_{app} \rightarrow k \cdot \frac{K_{eq}}{K_{eq} + \left(\frac{K^d'}{K^d}\right)^{\frac{1}{m}}}
\]

Receptor binding to either the closed or open capsid state favors the equilibrium toward the open state in equation 12 (diminishes \( k^f \) and increases \( k^r \)), but in equation 13, each state is favored by receptor binding to the sites available to that state (both rate constants are diminished) and the equilibrium is shifted toward the state with the higher-affinity binding due to the difference in the relative affinities between states. The \( k^r/k^f \) model in equation 12 does not argue against stabilization of the closed conformation when the receptor is bound (i.e., diminishing the influence of \( k^f \)), only that the augmentation of \( k^r \) predominates.

The simple cooperative model (Table 1, row B) showed half-maximal \( k_{app} \) at 48 nM receptor, and the \( k^r/k^f \) and MWC models (Table 1, rows E and F) gave best-fit values for the low-affinity equilibrium dissociation constants of 44 nM and 41 nM, respectively. While these are indirect measures based on best-fit models, all experiments were done at 37°C and direct measurements at 37°C have not been possible. Consequently, the \( K^d \) and \( K^d' \) values calculated here correspond to receptor concentrations for which the models fit the data (\( R, k_{app} \)), and the \( K^d \) values do not necessarily correspond to values of \( R \) where half of the total binding sites are occupied. If the half-maximal effects on \( k^f \) or \( k^r \) (equations 9 and 11) occur at occupancies other than 30 sites per capsid, then the calculated \( K^d \) or \( K^d' \) is different than the true equilibrium dissociation constants. If \( F \) is the fraction of total sites occupied that gives the half-maximal effect, rather than half-maximal binding, then the true \( K^d \) values will differ from those in Table 1 by the factor \( (1 - F)/F \). In other words, only if \( F \) is equal to 0.5 will the \( K^d \) and \( K^d' \) values in Table 1 correspond to equilibrium dissociation constants. Direct measurements of interactions between PV, HRV, and CVB and their receptors at 20°C have produced \( K^d \) values from 200 nM (a single \( K^d \) reported for PV-PVR (16)) to 12 μM (a low-affinity \( K^d \) for HRV3-ICAM-1 (15)). From the studies that reported both high- and low-affinity binding sites, the low-affinity and high-affinity equilibrium dissociation constants differed 7-fold (PV-PVR (17)) to 17-fold (HRV16-ICAM-1 (16)). Although the calculated \( K^d \) values in this analysis (data obtained at 37°C) were smaller than those previously determined by direct binding (at 20 to 25°C), the ratio, \( K^r/K^f \), from the \( k^r/k^f \) model was 7.3 and from the MWC model was 1.1. The relative difference between \( K^d \) and \( K^d' \) from the \( k^r/k^f \) model is more consistent with the published direct measurements but speaks to a need for careful study of temperature-dependent binding that might be extrapolated to physiological temperatures. One study did extrapolate data for the single \( K^d \) (versus temperature) found for PV-PVR and calculated a \( K^d \) of 80 nM at 37°C (16), not so different from the values calculated here for CVB3-sCAR. Studies that repeated binding experiments at different temperatures found increased affinities at higher temperatures (all ≤25°C) and reported that binding is endothermic (16, 17). Considering that capsid breathing is temperature dependent and likely to be enthalpy driven, perhaps the endothermic component of receptor binding may be attributed to the energy required for capsid transition from the closed to the open conformation that exposes the high-affinity site. By favoring capsids in the higher-energy conformation, the net effect of receptor binding would be endothermic due to the energy locked into the open capsid conformation.

The hyperbolic models (Table 1, row A; Fig. 1A) and the receptor-as-wedge model (Table 1, row D; Fig. 1D) that were previously considered (9, 16, 20) have been rejected on statistical grounds, as has the model in which the receptor has no effect on the conformational equilibrium (Table 1, row C; Fig. 1C). The simple cooperative model (Table 1, row B) provides an adequate empirical description of the relationship between \( k_{app} \) and the receptor in the CVB3/28-sCAR experimental system but fails to account for the capsid conformational equilibrium or the presence of receptor binding sites with different affinities and is rejected in favor of
models that account for these known mechanistic details. The $k' / k_0'$ (equation 12) and MWC (equation 13) models (Table 1, rows E and F) incorporate both the equilibrium between the closed and open conformations and different respective binding constants (i.e., they consider capsid breathing). Fundamentally, both models present the same conclusion: binding receptor to a high-affinity site shifts the equilibrium to the conformation in which the high-affinity site is available. Figure 7 illustrates the relative abundances of the equilibrium species present in the scheme of Fig. 4, calculated using the relationships in Fig. 5 with $k' _C$ and $k' _O$ from equations 9 and 11 (model E in Table 1). The plot clearly shows a shift from the closed (V, VR) conformation to the open (U, UR) conformation as receptor concentration increases, as well as the rise and fall of VR as UR increasingly dominates the population. Recalling that $dA/dt$ is equal to $k \cdot (U + UR)$, this plot illustrates how the receptor can increase the velocity at which the virus is converted to A-particles by increasing UR. This is allostery by conformation selection, so the receptor does not trigger virus conversion to A-particles but, by stabilizing the more energetic open-conformation intermediate, increases the probability that the virus will transition to A-particles.

Acceptance of published observations and some assumptions have been required to justify the allosteric models, the foremost of which are the existence of the conformational equilibrium (breathing) and the two conformation-dependent receptor binding sites. Multiple studies have documented conformational differences in HRV and PV (and other viruses) that are demonstrable at 37°C but not at lower temperatures (e.g., 20 to 25°C) (5–10). Distinct conformation-dependent receptor binding sites on PV and HRV are supported by four studies, three of which inferred distinct $K_d$ values from best fits to receptor binding curves measured by plasmon resonance (at <30°C) and one of which (a structural study) captured a population of HRV3-ICAM-1 complexes generated at 37°C (9, 15–17). However, one study that found two conformation-dependent receptor binding sites for HRV detected only a single $K_d$ for PVR binding to PV. Also, note that the temperatures at which the temperature-dependent high-affinity sites were observed (<30°C) are temperatures at which capsids have been reported to be impermeable to alkylation agents and at which Vp1 and Vp4 peptides characteristic of the open conformation were not detectable (i.e., they should be in the closed conformation) (5, 8). Receptor-catalyzed conversion of the virus to A-particles increased exponentially for HRV3 as temperature increased above 30°C and was 81-fold faster for PV at 37°C than at 29°C, reinforcing the likelihood that observations limited to temperatures below 30°C miss important details available only near physiological temperatures (14, 21). Perhaps these large differences between observations made at 37°C and those made at lower temperatures will eventually explain why evidence of cooperativity predicted by the allosteric models is not present in the published Scatchard plot of PV-binding PVR (17). So, while the conformational equilibrium at 37°C appears to be established and the receptor binding site(s) available at lower temperatures has been reasonably well characterized, knowledge about the receptor binding site available at 37°C has not been as accessible.

Accepting the existence of a conformational equilibrium and two conformation-dependent receptor binding sites, an allosteric model should be the most appropriate for describing the equilibria, and the kinetics, in these virus-receptor systems (22). With the assumptions that a single first-order rate constant, $k$, applies to the open conformation(s) (U and UR), that the open conformation is intermediate in the pathway from the closed conformation (V and VR) to A-particles, and that the conversion to A-particles is the rate-limiting step, the allosteric models describe the relationship between $k_{op}$ and soluble CAR for the receptor-catalyzed conversion of CVB3/28 to A-particles. The models should apply to each of the considered virus-receptor systems, with the note that values for $K_{eq}$ and $k$ are expected to vary among virus species and strains and be susceptible to effects of ligands that alter the conformational dynamics or the transition from the open conformation to A-particles.

Why would evolution favor capsid binding to receptors via an essentially nonfunctional low-affinity mechanism in addition to binding to a conformation-dependent high-affinity site when the high-affinity site alone would suffice for both binding to the target cell and catalysis of the conversion to A-particles? One possible answer was provided by the observations that low-affinity binding to secondary ligands, such as CD55, can serve to anchor the virus to cells and transduce signals that facilitate viral access to a seques tered receptor (24) and that viruses anchored to cells by such ligands may allow time for them to acquire sufficient numbers of receptors to support infection (25). Similar arguments may be relevant to low-affinity receptor binding, especially for strains that do not bind secondary ligands. Viruses bind target cells that express anchored receptors (or ligands) often available in ample numbers. Once the virus is bound to the cell, receptors that disso ciate from the bound virus are much more likely to reengage the virus still attached to nearby possibly clustered receptors than are soluble receptors that are comparably free to diffuse away from the virus. So, even low-affinity binding to multiple receptors can produce high-avidity capture of the virus at the cell surface, and if the low-affinity binding involves the receptor that can also bind the high-affinity site, then virus association with a legitimate target cell has also been facilitated. If viruses bind low numbers of receptors and become inactivated, cellular shedding or secretion...
of soluble receptors would be a useful innate defensive mechanism by which to neutralize viruses in tissue fluids. Such cellular secretion or shedding of soluble forms of receptors, documented for CAR (26, 27), ICAM-1 (28), and PVR (29), could select for the low-affinity/high-avidity-binding viruses. With $K_d$ for the closed and open conformations of CVB3/28 near 0.05, the capsids are in the closed (low-affinity) conformation about 95% of the time. In tissue fluids, especially those where there is some turbulence, the chances of a soluble receptor engaging a high-affinity site would be low, and the chances for engagement by multiple soluble receptors even smaller. In contrast, low-affinity/high-avidity binding at the cell surface increases the likelihood that the capsid will remain engaged with receptors until the open conformation becomes available, allowing the receptors to catalyze the transition to A-particles on the pathway to infection. In this context, the viruses have adapted to decoy receptors shed by target cells by developing decoy low-affinity receptor binding sites that remain useful for discriminating between free and cell-associated receptors. The low-affinity binding that facilitates host cell targeting and evasion of inactivation by soluble receptors combined with the availability of the catalytic high-affinity receptor binding site in an appropriately warm host speaks to the evolutionary fine-tuning of these pathogens.

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REFERENCES