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The structure of antiviral agents that inhibit uncoating when complexed with viral capsids

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Summary

The tertiary structure of most icosahedral viral capsid proteins consists of an eight-stranded antiparallel β-barrel with a hydrophobic interior. In a group of picornaviruses, this hydrophobic pocket can be filled by suitable organic molecules, which stop viral uncoating after attachment and penetration into the host cell. The antiviral activity of these agents is probably due to increased rigidity of the capsid protein, thereby inhibiting disassembly. The hydrophobic pocket may be an essential functional component of the protein and may have been conserved in the evolution of many viruses from a common precursor. Since eight-stranded antiparallel β-barrels, with a topology as in viral capsid proteins, are not generally found in other proteins involved in cell metabolism, antiviral agents that bind in the interior of viral capsid proteins are likely to be more virus-specific and less cytotoxic. Furthermore, the greatest conservation of viral capsid proteins occurs within this pocket, whereas the least conserved part is the antigenic exterior. Thus, compounds that bind to such a pocket are likely to be effective against a broader group of serologically distinct viruses. Discovery of antiviral agents of this type depends on designing compounds that can enter and fit snugly into the hydrophobic pocket of a particular viral capsid protein.

Hydrophobic pocket; Viral capsid protein; Antiparallel β-barrel; Inhibition of uncoating

Introduction

Because many spherical viruses can frequently form beautiful single crystals, the three-dimensional atomic structures of a few of these viruses have been deter-

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TABLE 1

Three-dimensional high resolution structures of spherical viruses

		Capsid[1] symmetry	Reference
Plant RNA viruses			
Tombus group	Tomato bushy stunt	$T = 3$	Harrison et al., 1978
	Turnip crinkle	$T = 3$	Hogle et al., 1986
Sobeamo group	Southern bean mosaic virus	$T = 3$	Abad-Zapatero et al., 1980
	Satellite tobacco necrosis	$T = 1$	Liljas et al., 1982
Como group	Cowpea mosaic virus	$P = 3$	Stauffacher et al., 1987
	Beanpod mottle virus	$P = 3$	J. Johnson, unpublished results
Animal RNA viruses			
Rhino-	Human rhino 14	$P = 3$	Rossmann et al., 1985
	Human rhino 1A	$P = 3$	S. Kim and M.G. Rossmann, unpublished results
Entero-	Polio Mahoney 1	$P = 3$	Hogle et al., 1985
	Polio Sabin 3	$P = 3$	J. Hogle, private communication
Cardio-	Mengo	$P = 3$	Luo et al., 1987
Aphtho-	Foot-and-mouth disease O1K	$P = 3$	D. Stuart, private communication
Insect RNA Viruses			
	Black beetle virus	$T = 3$	Hosur et al., 1987

[1] $T = N$ ($N = 1, 3, 4, 7...$) relates to the triangulation number (Caspar and Klug, 1962).
$P = 3$ implies a pseudo $T = 3$ surface lattice arrangement when there are three non-identical but similarly folded protein subunits arranged as in a $T = 3$ lattice.

mined during the last decade (Table 1) using X-ray crystallography. This has been possible due to advances in instrumentation, the availability of synchrotrons for the production of very intense X-rays, the advent of powerful supercomputers as well as advances in techniques used for the analysis of the X-ray data (Arnold et al., 1987). Although at this time the available structures are only those of simple RNA viruses with a radius of less than 320 Å, it can be anticipated that there will be an increasing rate of new information on virus structures of all kinds including DNA viruses (Luo et al., 1988; Roberts et al., 1986; Rayment et al., 1982) and enveloped viruses (Wilson et al., 1981; Colman et al., 1983; Fuller, 1987). The most unexpected and surprising aspect of these structures is that there is a remarkable

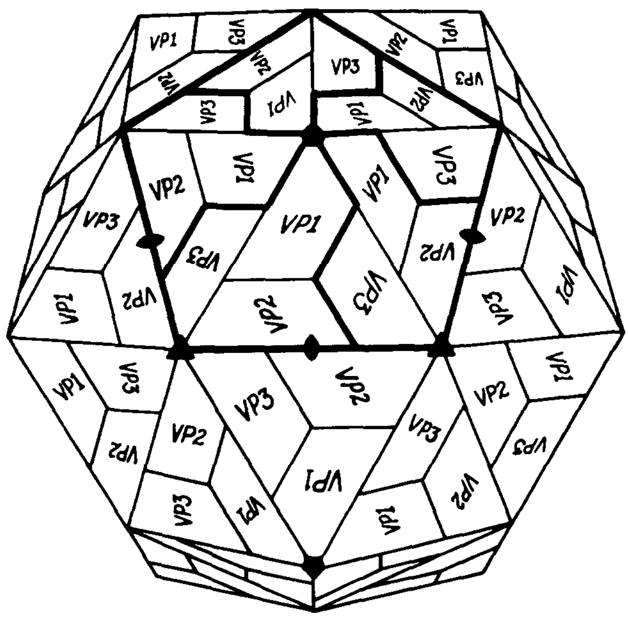

Fig. 1. Icosahedral capsid: the thickly outlined VP1, VP3 and VP2 unit corresponds to the 6S (VP1, VP3, VP0) promoter and the 15-mer cap to the 14S pentamer observed in assembly experiments.

similarity among them. Their quarternary structural organization of protein subunits is essentially the same (Fig. 1) as is also the tertiary polypeptide fold of each protein (Fig. 2). Even in the larger, DNA-containing adenovirus, the hexon unit of the capsid (Roberts et al., 1986) contains a tandem repeat of the eight-stranded antiparallel β-barrel fold (Fig. 2) found in the RNA viruses.

It is a relatively simple extension of the crystallographic technique to examine complexes of these viruses with small ligands that alter the properties and modify the structure of the viral capsid. In particular, it has been possible to examine the mode of binding of a series of compounds that inhibit viral uncoating of rhino- and enteroviruses (Badger et al., 1988a; Smith et al., 1986).

There are a variety of compounds that can inhibit disassembly while interfering little or not at all with viral attachment or penetration of the host cell membrane (McSharry et al., 1979; Diana et al., 1977, unpublished results; Ninomiya et al., 1984; Lonberg-Holm et al., 1975). Mutants that are resistant to a compound in a specific rhinovirus have altered activity to other compounds (D. Tyrrell and F.J. Dutko, private communication; Eggers and Rosenwirth, 1988), showing that these compounds probably all bind to essentially the same site in picornaviruses. The in vitro minimal inhibitory concentrations (MIC) to reduce plaque counts by 50% can be as low as 0.02 μM depending on the specific virus and compound. Their range varies greatly, but, for instance, 0.25 μg/ml of a dichloro derivative of compound VI (Fig. 3) inhibited 80% of 53 rhinovirus serotypes that were tested. The in vivo activity of the same compound in suckling mice is shown in Fig. 4.

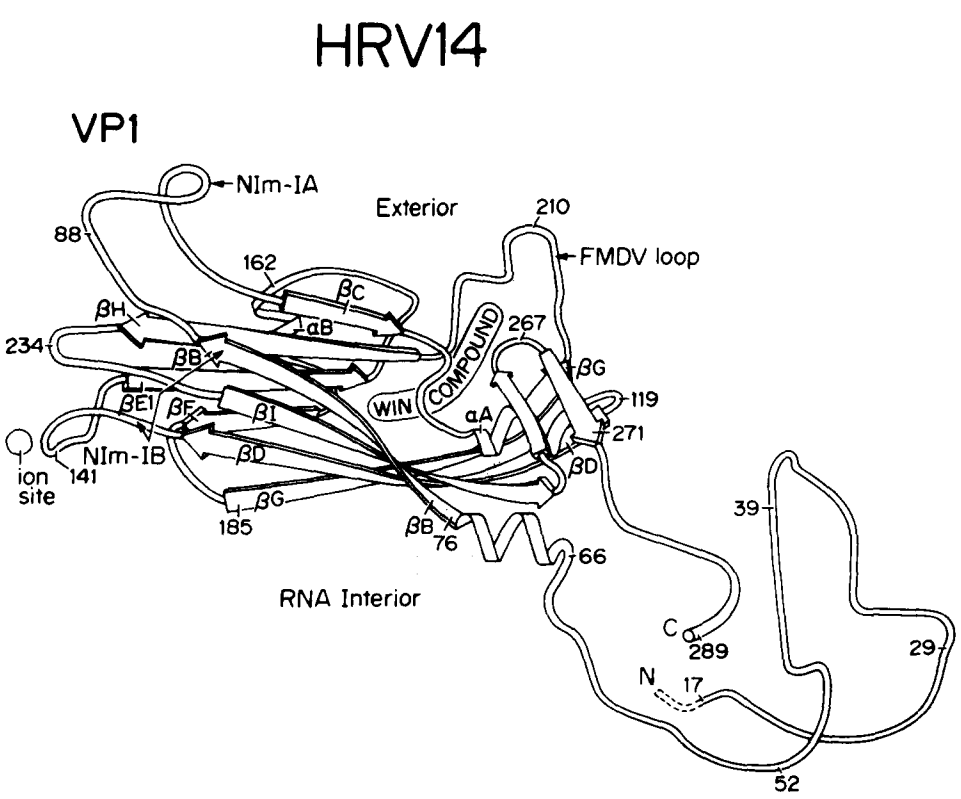

Fig. 2. The eight-stranded antiparallel β-barrel, as found in viral capsid protein 1 (VP1) of human rhinovirus, is the site of attachment of antiviral WIN compounds. They bind into the hydrophobic internal pocket as shown. NIm-IA and NIm-IB are hypervariable, external neutralizing immunogenic sites on HRV-14. Secondary structural elements (βB, βC..., αA, αB, FMDV loop) and approximate sequence numbers are shown.

Structure

The site of binding of a series of 'WIN' compounds (Fig. 3), synthesized by the Sterling-WINthrop Research Institute, to human rhinovirus 14 (HRV-14) and 1A (HRV-1A) have been examined crystallographically. The two serotypes of HRV were selected because they bind to different HeLa cell receptors (Abraham and Colonno, 1984). All of the WIN compounds were located at the same general site in the hydrophobic pocket within the eight-stranded antiparallel β-barrel of VP1 (Fig. 5). This pocket can be entered by way of a pore on the floor of a surface depression ('canyon') which is almost certainly the site of receptor attachment (Rossmann et al., 1985; Rossmann and Palmenberg, 1988; Colonno et al., 1988). The compounds with a seven-membered aliphatic chain and an aliphatic group (methyl or ethyl) on the oxazoline group that have been examined thus far bind in one orientation (Fig. 6A), while the other compounds bind in the opposite di-

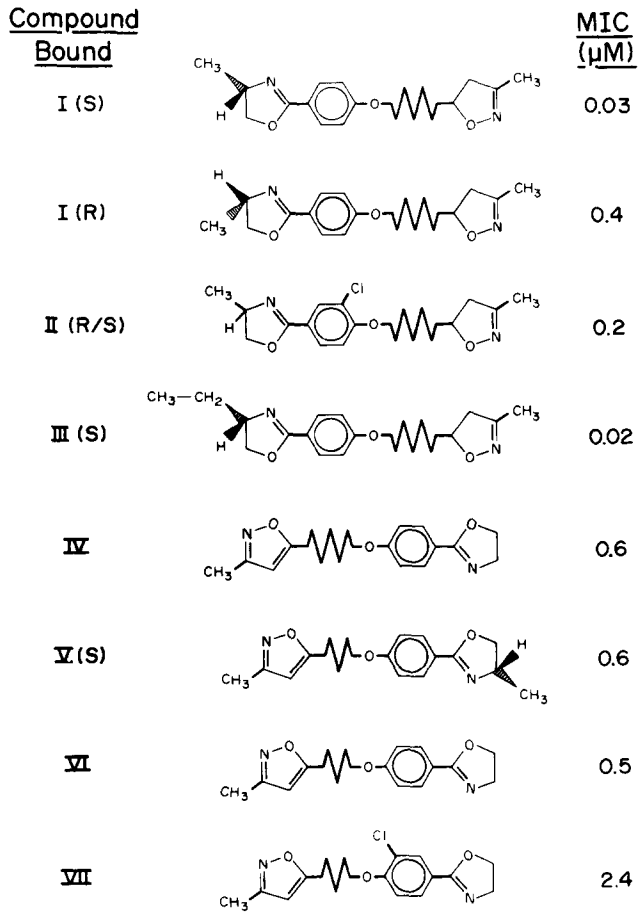

Fig. 3. Formulae of antiviral compounds examined on binding to HRV-14. Compound VII has been examined when binding to HRV-14 as well as HRV-1A. Shown also are the in vitro activities against HRV-14 measured in terms of the concentration (μM) required to reduce the plaque counts by an MIC factor of two.

rection (Fig. 6B). The occupancy of the compounds is roughly 60% in the crystal (that is, almost two-thirds of the 60 sites in each virion are occupied), probably depending on the length of time of the crystal soak, the type of compound and its solubility. Binding studies on the virus in solution show a somewhat higher occupancy (F.J. Dutko and T.J. Smith, unpublished results).

The pocket entrance of HRV-14 is partially blocked by Met 221 of VP1. This is pushed out of the way by the presence of the WIN compounds, causing a 4 Å movement of the 'FMDV loop' (Figs. 2 and 7) and some other smaller changes as well. These sizeable conformational changes are always the same, independent of the compound and its mode of binding. However, the structure of native HRV-1A shows the pocket already open (S. Kim, M.S. Chapman and M.G. Rossmann,

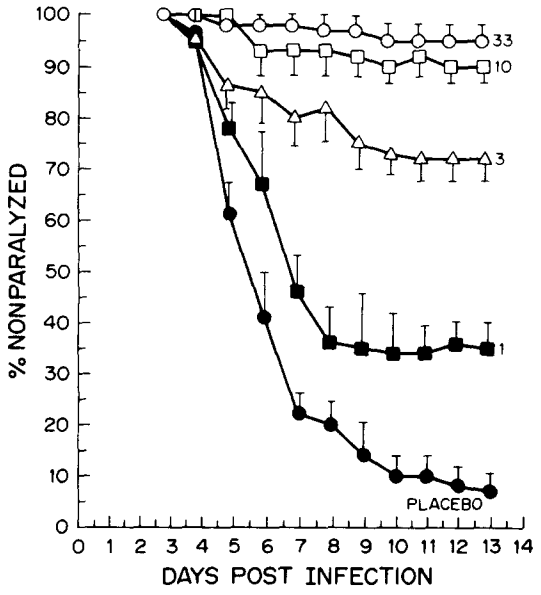

Fig. 4. Therapy with a WIN compound against coxsackie A9 virus in suckling mice. The latter were treated once a day for 5 days, The curves show the effect of different relative dosages in mg/kg of mouse/day.

unpublished results). Indeed, the structure of HRV-1A resembles far more that of HRV-14 with a bound WIN compound. Thus, no significant conformational change occurs on binding compound VII to HRV-1A. In Mengo virus, entrance to the pocket is blocked by a large insertion in VP1. Furthermore, those WIN compounds that have been tested have no effect on Mengo virus.

Compounds I, III and V (Fig. 3) have a chiral carbon atom on the oxazoline ring. The *S* optical isomer has considerably greater efficacy in each case. It was, therefore, of interest that on diffusing a racemic mixture into crystalline HRV-14,

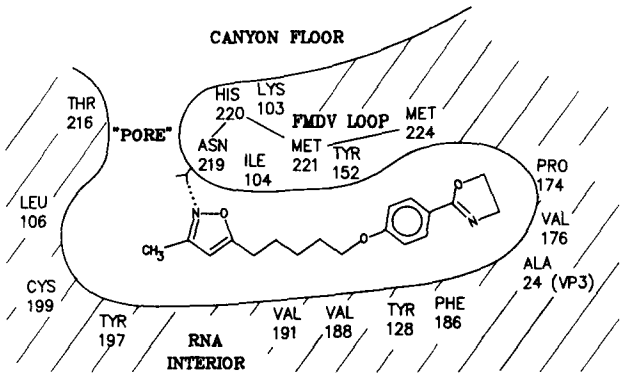

Fig. 5. Diagrammatic representation of compound VI bound in the WIN pocket. Shown also are the residues lining the pocket. The orientation is opposite to that of compound I (Smith et al., 1986).

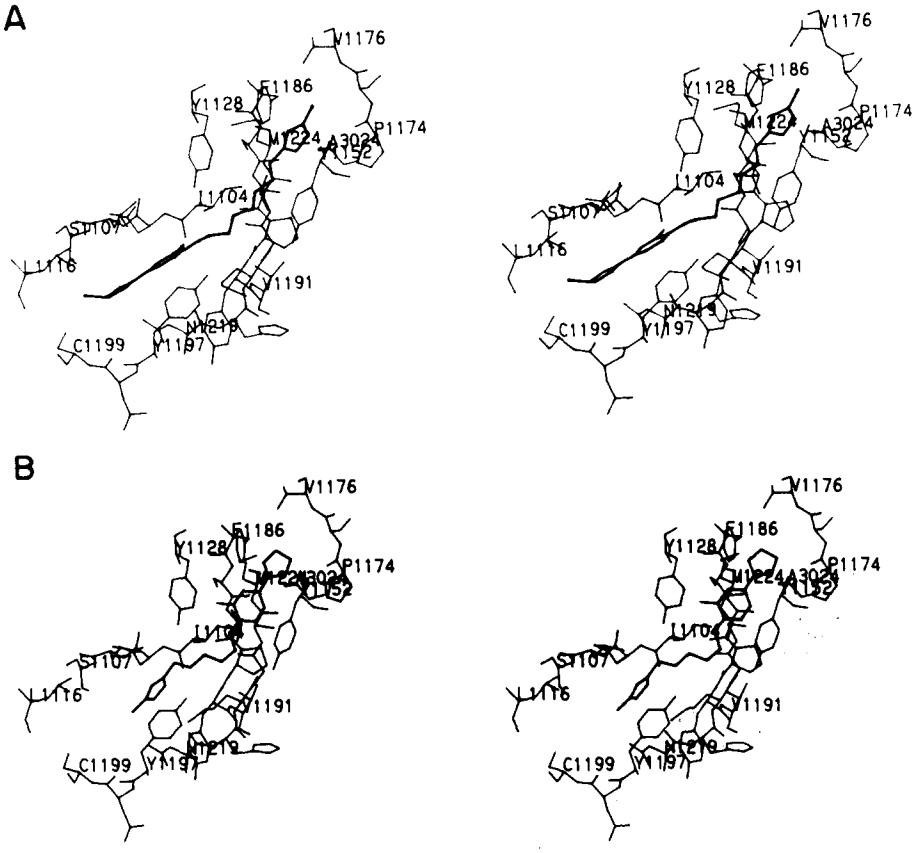

Fig. 6. Protein environment of bound compound II (A) and compound VII (B). Both of these compounds contain certain chlorine atoms, but they differ in the length of their alipathic chain, the presence of a methyl substituent on the oxazoline group of compound II and in their orientation within the binding pocket.

only the *S* isomer was seen to bind. This observation as well as binding measurements have shown that the efficacy of these compounds (MIC) is closely correlated to their ability to bind to the virus.

Drug-resistant mutants

A series of drug-resistant mutants have been selected (B. Heinz and R. R. Rueckert, in preparation) by growing the virus in the presence of high concentrations of compound I(*S*) or IV. Most of the mutations altered residues lining the WIN binding pocket (Fig. 5). Particularly frequent were mutations of residue Val 188 and Cys 199 in VP1. In each case, the resistant mutant was a larger residue. The structures of V188→L and C199→Y have been examined crystallographically

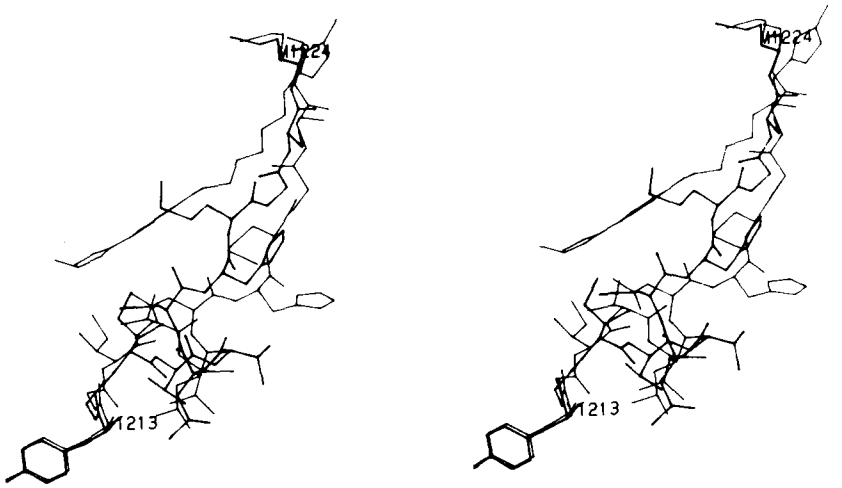

Fig. 7. Superposition of native HRV-14 (heavy line) and the viral conformation altered by drug binding (light line): residues 1213–1224.

(Badger et al., 1988a, and unpublished results). The results show that resistance arises from steric hindrance, stopping complete insertion of the compound into the pocket. Differences in the MIC values with respect to the mutated (V188→L) virus showed some correlation with the direction of binding of the compounds in the WIN pocket. Those compounds (I–III) which would have their aliphatic chains opposed to the mutant leucine had lower MIC values than the other compounds. In the case of the C199→Y mutation, a good deal of the structural environment was also changed and this has been successfully 'predicted' using molecular dynamics calculations (Badger et al., unpublished results).

Mechanism of uncoating inhibition

Two possible mechanisms for the activity of the WIN compounds have been suggested (Smith et al., 1986): (a) binding of the compounds might inhibit the flow of ions through the WIN pocket into the virus interior, which would cause swelling and disassembly; and (b) binding of the compounds would stiffen VP1 and, therefore, inhibit the necessary flexibility for uncoating. Although the diffusion of heavy metal ions into virus interiors does occur (Harrison et al., 1978), analysis of the refined HRV-14 structure does not reveal any special channels (Rossmann and Palmenberg, 1988). Furthermore, the interior of the pocket, where the $n = 5$ compounds bind, is not an optimal site for stopping ion flow. Thus, the ion-flow hypothesis now seems less probable. On the other hand, hypothesis (b) has some confirmation in light of the observation that the SW12 mutant (Val 1188→Leu) is significantly more resistant to thermal inactivation in the presence of drugs than is wild-type virus (F.J. Dutko, unpublished results). Thus, filling the pocket with WIN compounds or WIN compounds and larger amino acid side chains can improve stability and, hence, decrease uncoated potential.

The presence of a potential hydrophobic pocket in numerous viral capsids is probably not fortuitous. Some degree of flexibility may be required to accommodate the assembly and disassembly process. This can be provided by the loosely packed internal hydrophobic pocket of the standard viral capsid protein. If this pocket is filled by a molecule of appropriate size and physical characteristics, then the protein becomes rigid and fails to perform its normal assembly and disassembly functions. Indeed, the requirement for this function in a protein that can also assemble into an icosahedral particle may, in part, be the cause for the retention of the same protein fold in the evolution of so many viral capsid structures. The shape and size of the hydrophobic pocket varies from one virus to another according to the particular amino acids that line the pocket. The pocket is not necessarily equally accessible in different viruses. For instance, Mengo virus (Luo et al., 1987) has a hydrophobic interior to VP1 but it is probably not accessible to WIN compounds. Similarly, the WIN compounds penetrate only into VP1, not into the homologously folded VP2 or VP3 of HRV. The design of a suitable antiviral agent that inhibits uncoating will thus depend on the knowledge of the precise structure of the targeted virus capsid protein. The agent must be sufficiently flexible to enter the pocket through an available pore on the exterior of the capsid, sufficiently hydrophobic to be retained by the pocket, and of suitable size to fit into the pocket.

The eight-stranded antiparallel β-barrel motif, with a topology as in viral capsid proteins, has not been found in other classes of proteins (Richardson, 1979). Thus WIN-like compounds, which have been particularly adapted to bind with high affinity to a specific viral capsid protein, are unlikely to bind with the same affinity to other types of proteins with different folds. This is a possible explanation for the limited toxicity for compounds of this class. In contrast, antiviral agents targeted at (for instance) viral proteases or polymerases have to have greater specificity in order not to interfere with essential metabolic processes that are dependent on proteins with similar functions and, therefore, probably also with a similar fold.

The greatest conservation between different picornaviruses occurs in the internal residues, whereas the greatest variability occurs on the antigenic surface (Rossmann and Palmenberg, 1988). Thus, antiviral agents such as the WIN compounds have a relatively large range covering not only most rhinoviruses but also many enteroviruses as well. The high surface variability (Rossmann et al., 1985) accounts for the large number of serologically distinct viruses which, nevertheless, bind to only a few different receptors (Colonno et al., 1986; Mapoles et al., 1985). This is on account of the partially hidden receptor attachment site in the canyon (Rossmann et al., 1985).

In light of the remarkable conservation of the eight-stranded antiparallel motif as the basic building unit of numerous RNA as well as DNA viruses, it would not seem improbable that the same structure occurs in the capsid protein of retroviruses (Argos and Fuller, 1988) and flaviviruses (Fuller and Argos, 1987). The hydrophobic character of WIN compounds is essential for their binding into the pocket within a β-barrel. Their hydrophobic character is also likely to allow them to be

absorbed and transported across viral membranes, or adsorbed on the capsid during assembly. Thus, variations of these compounds might be as useful to inhibit uncoating of enveloped viruses such as flaviviruses (e.g. Sindbis virus) or human immunodeficiency virus as they are in inhibiting simple icosahedral viruses.

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Note added in proof: Since this paper was first written, Pevear et al. (1989) have shown that the formation of complexes between WIN compounds and HRV14, but not HRV1A, inhibits attachment to cell membranes. Thus, the conformational changes caused by WIN binding to HRV14 in the floor of the canyon are directly correlated with the ability of the virus to attach. In HRV1A, where WIN compounds do not cause such conformational changes, there is no inhibition of attachment.

Acknowledgement

Figures have been reprinted with permission, Rossmann et al., 1985 (Fig. 1); Luo et al., 1987 (Fig. 2); Badger et al., 1988 (Figs. 3, 6, 7); McKinlay et al., to be published in J. Med. Chem., March 1989 (Fig. 4). Fig. 5 has been derived from Smith et al., 1986.