Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 4625–4627, July 1988
Biochemistry

Antiviral agents targeted to interact with viral capsid proteins and a possible application to human immunodeficiency virus

(conserved antiviral target/viral uncoating/capsid structure/icosahedral viruses)

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ABSTRACT **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 thereby 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, which inhibits disassembly. The hydrophobic pocket may be an essential functional component of the protein and, therefore, may have been conserved in the evolution of many viruses from a common precursor. Since eight-stranded anti-parallel β-barrels, with a topology as in viral capsid proteins, are not generally found for other proteins involved in cell metabolism, this class of antiviral agents is 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 will, therefore, depend on designing compounds that can enter and fit snugly into the hydrophobic pocket of a particular viral capsid protein. The major capsid protein, p24, of human immunodeficiency virus would be a likely suitable target.**

General

Atomic-resolution structures of a few icosahedral RNA plant and animal viruses have been determined in the last decade (Table 1). In each case the essential shell domain consists of an eight-stranded antiparallel β-barrel (Fig. 1). In general, the quaternary organizations of the plant and animal virus icosahedral shells are also remarkably alike. The characters of the three-dimensional viral structures are sufficiently similar to make it most probable that many simple spherical RNA viruses have evolved from a common precursor (9, 12). The similarity in folding topology extends to the more complex icosahedral DNA adenoviruses (11) and to the bacillus-shaped RNA alfalfa mosaic virus (ref. 6; I. Fita and M.G.R., unpublished results). In the latter case, the viral capsid consists of a cylindrical capsid capped with a hemi-icosahedron at either end. The length of these capsids is dependent on the size of the RNA encapsidated. The protein subunits, under suitable conditions, can reassemble into complete icosahedra. It has also been shown that the core protein of Sindbis virus is homologous to VP3 of foot-and-mouth disease virus and may, therefore, also have the same tertiary and quaternary structure (13). Sindbis virus has a lipid envelope with an internal nucleocapsid core somewhat similar to retroviruses.

Table 1. Viruses whose capsid proteins are similarly folded and assembled

Virus	Capsid Symmetry	Capsid Diameter, Å	Ref.
Plant RNA viruses			
Tomato bushy stunt virus	$T=3$	350	1
Southern bean mosaic virus	$T=3$	300	2
Satellite tobacco necrosis virus	$T=1$	190	3
Turnip crinkle virus	$T=3$	350	4
Cowpea mosaic virus	$P=3$	300	5
Alfalfa mosaic virus	Bacillus	190	6, *
Animal RNA viruses			
Human rhinovirus 14	$P=3$	300	7
Poliovirus	$P=3$	300	8
Mengo virus	$P=3$	300	9
Insect RNA virus			
Black beetle virus	$T=3$	300	10
Animal DNA virus			
Adenovirus hexon	—	—	11

T (triangulation) = 3 implies that there are 3 × 60 capsid proteins in the icosahedral viral shell. P (pseudo) = 3 implies that there are 60 copies of each of 3 different major proteins in the icosahedral viral shell. However, the fold of the three different poplypeptides chains is similar.
*, I. Fita and M.G.R., unpublished results.

A variety of antiviral agents that inhibit viral uncoating have been reported for the rhino- and enterovirus members of the picornavirus family (14–17). WIN 51711 is representative of a class of compounds that inhibit picornavirus replication in tissue culture (18) and in animal models of human enterovirus disease (19, 20). The binding site for WIN 51711, as well as a variety of related compounds, on the viral capsid of HRV14 has been examined by using x-ray crystallography (21, 22). These compounds bind to the hydrophobic interior of VP1 and HRV14, causing significant conformational changes in enlargement of the pocket within the viral capsid. They stabilize the capsid by making the coat protein sufficiently rigid to prevent the capsid proteins from disengaging from each other during disassembly.

The presence of a potential hydrophobic pocket in the typical viral capsid 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 (e.g., WIN 51711), then conformational changes are induced and 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

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Abbreviations: VP, viral capsid protein; HRV14, human rhinovirus 14; HIV, human immunodeficiency virus.

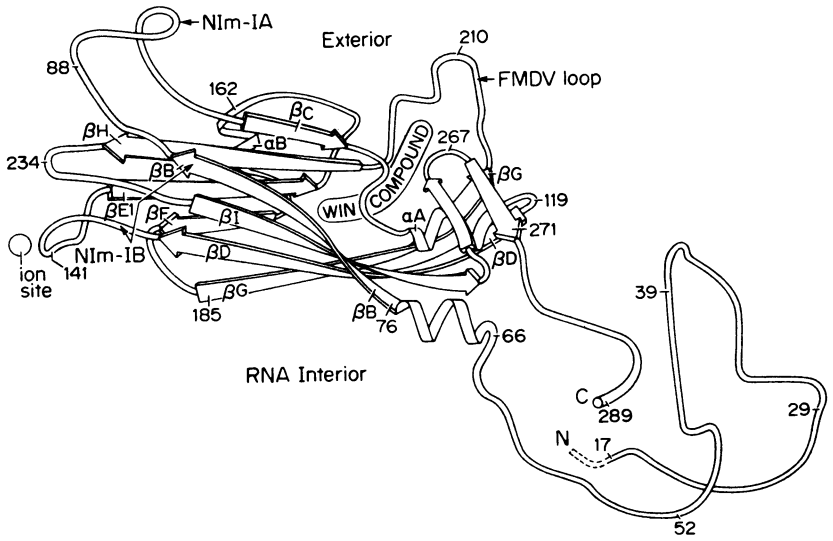

FIG. 1. The eight-stranded antiparallel β-barrel as found in viral capsid protein 1 (VP1) of human rhinovirus 14 (HRV14). The site of attachment of antiviral agent WIN 51711 into the hydrophobic internal pocket is shown. NIm-IA and NIm-IB are neutralizing immunogenic sites. Secondary structural elements (βB, βC, . . ., αA, αB, FMDV loop) and approximate sequence numbers are shown.

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 (9) has a hydrophobic interior to VP1 but it is not readily accessible and WIN 51711 is not active in this virus. Similarly, the WIN compounds penetrate only into VP1, not into the homologously folded VP2 or VP3 of HRV14. 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 capsid's exterior, sufficiently hydrophobic to be retained by the pocket, and of suitable size to fit into the pocket. Drug design can further be aided by experiments with well-chosen compounds.

The eight-stranded antiparallel β-barrel motif with a topology as in viral capsid proteins has not been found in other classes of proteins (23). Thus compounds like WIN 51711, 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 seen with 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 function 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 (24). The high surface variability (7) accounts for the large number of serologically distinct viruses, which, nevertheless, bind to only a few different receptors (25, 26). Thus, antiviral agents such as WIN 51711 have a relatively large range covering not only most rhinoviruses but also many enteroviruses as well.

The hydrophobic character of compounds such as WIN 51711 is essential for their binding to the hydrophobic pocket in VP1 of picornaviruses. However, their hydrophobic character is likely to allow them to be absorbed and transported across viral membranes. They could also be adsorbed on the capsid during assembly. Thus, variations of these compounds might be as useful to inhibit uncoating of enveloped viruses such as Sindbis virus or human immunodeficiency virus (HIV) as they are in inhibiting simple icosahedral viruses.

Application to HIV

The major capsid protein for HIV is p24, a post-translationally modified *gag* gene product. The other products are p13 and p18. The exceedingly basic character of p13 suggests that it might be associated with the viral RNA (27), as is also common with plant viruses (1, 2). The p18 protein is probably associated with the HIV membrane. In HIV the p24 protein forms a bacillus- or cone-shaped capsid (28) containing about (2 × 9600) RNA bases. By proportionality with similarly shaped bacillus-shaped particles of alfalfa mosaic virus (6, 29), the p24 core capsid could have icosahedral ends with $T = 3$ symmetry (30), a 300-Å diameter, and a 950-Å-long cylindrical central component. Not only would such an envelope be of sufficient size to contain the RNA, but also it does correspond roughly with observed dimensions.

Coates *et al.* (31) predict that the secondary structure of p24 is mainly α-helical. However, the 24,000 molecular weight of p24 roughly corresponds to the size of a standard viral capsid protein. For instance, the molecular weights of VP1, VP2, and VP3 in picornaviruses ranged from 24,000 to 35,000. While viral capsid proteins derived from different viruses (e.g., picornaviruses and plant viruses) generally have similar three-dimensional structures, they do not show any obvious amino acid sequence equivalence. Yet "fingerprints" determined from the alignments of diverse RNA capsid proteins can also be detected in HIV p24 (Ann C. Palmenberg, personal communication).

In light of these properties of HIV p24 and the observations that many virus capsid proteins have similar tertiary structures and have similar capsid organizations, it is probable that p24 has the eight-stranded anti-parallel β-barrel structure typical in other viruses. Hence, p24 might contain a hydrophobic pocket and would be a suitable target for antiviral compounds that would inhibit viral uncoating. The efficacy of these compounds is less likely to be affected by rapid change in amino acid sequences of the p24 protein as a result of immune surveillance.

I am indebted to numerous stimulating discussions with many of my colleagues, in particular, Mark McKinlay at Sterling–Winthrop Research Institute, and also Tom Smith at Purdue University, Guy Diana and Frank Dutko at Sterling–Winthrop Research Institute, and Ann Palmenberg, Roland Rueckert, and Beverly Heinz at the University of Wisconsin. I am also grateful to Sharon Wilder for help in preparing this manuscript. The results on which this paper is based were obtained with the help of grants from the National Institutes of Health and the National Science Foundation.

1. Harrison, S. C., Olson, A. J., Schutt, C. E., Winkler, F. K. & Bricogne, G. (1978) *Nature (London)* **276,** 368–373.
2. Abad-Zapatero, C., Abdel-Meguid, S. S., Johnson, J. E., Leslie, A. G. W., Rayment, I., Rossmann, M. G., Suck, D. & Tsukihara, T. (1980) *Nature (London)* **286,** 33–39.
3. Liljas, L., Unge, T., Jones, T. A., Fridborg, K., Lövgren, S., Skoglund, U. & Strandberg, B. (1982) *J. Mol. Biol.* **159,** 93–108.
4. Hogle, J. M., Maeda, A. & Harrison, S. C. (1986) *J. Mol. Biol.* **191,** 625–638.
5. Stauffacher, C. V., Usha, R., Harrington, M., Schmidt, T., Hosur, M. V. & Johnson, J. E. (1987) in *Crystallography in Molecular Biology*, eds. Moras, D., Drenth, J., Stranberg, B., Suck, D. & Wilson, K. (Plenum, New York), pp. 293–308.
6. Fukuyama, K., Abdel-Meguid, S. S., Johnson, J. E. & Rossmann, M. G. (1983) *J. Mol. Biol.* **167,** 873–894.
7. Rossmann, M. G., Arnold, E., Erickson, J. W., Frankenberger, E. A., Griffith, J. P., Hecht, H. J., Johnson, J. E., Kamer, G., Luo, M., Mosser, A. G., Rueckert, R. R., Sherry, B. & Vriend, G. (1985) *Nature (London)* **317,** 145–153.
8. Hogle, J. M., Chow, M. & Filman, D. J. (1985) *Science* **229,** 1358–1365.
9. Luo, M., Vriend, G., Kamer, G., Minor, I., Arnold E., Rossmann, M. G., Boege, U., Scraba, D. G., Duke, G. M. & Palmenberg, A. C. (1987) *Science* **235,** 182–191.
10. Hosur, M. V., Schmidt, T., Tucker, R. C., Johnson, J. E., Gallagher, T. M., Selling, B. H. & Rueckert, R. R. (1987) *Proteins* **2,** 167–176.
11. Roberts, M. M., White, J. L. Grütter, M. G. & Burnett, R. M. (1986) *Science* **232,** 1148–1151.
12. Rossmann, M. G. & Rueckert, R. R. (1987) *Microbiol. Sci.* **4,** 206–214.
13. Fuller, S. D. & Argos, P. (1987) *EMBO J.* **6,** 1099–1105.
14. McSharry, J. J., Caliguiri, L. A. & Eggers, H. J. (1979) *Virology* **97,** 307–315.
15. Diana, G. D., Salvador, U. J., Zalay, E. S., Johnson, R. E., Collins, J. C., Johnson, D., Hinshaw, W. B., Lorenz, R. R., Thielking, W. H. & Pancic, F. (1977) *J. Med. Chem.* **20,** 750–756.
16. Ninomiya, Y., Ohsawa, C., Aoyama, M., Umeda, I., Suhara, Y. & Ishitsuka, H. (1984) *Virology* **134,** 269–276.
17. Lonberg-Holm, K., Gosser, L. B. & Kauer, J. C. (1975) *J. Gen. Virol.* **27,** 329–342.
18. Otto, M. J., Fox, M. P., Fancher, M. J., Kuhrt, M. F., Diana, G. D. & McKinlay, M. A. (1985) *Antimicrob. Agents Chemother.* **27,** 883–886.
19. McKinlay, M. A. & Steinberg, B. A. (1986) *Antimicrob. Agents Chemother.* **29,** 30–32.
20. McKinlay, M. A., Frank, J. A. & Sternberg, B. A. (1986) *J. Infect. Dis.* **154,** 676–681.
21. Smith, T. J., Kremer, M. J., Luo, M., Vriend, G., Arnold, E., Kamer, G., Rossmann, M. G., McKinlay, M. A., Diana, G. D. & Otto, M. J. (1986) *Science* **233,** 1286–1293.
22. Badger, J., Minor, I., Kremer, M. J., Oliveira, M. A., Smith, T. J., Griffith, J. P., Guerin, D. M. A., Krishnaswamy, S., Luo, M., Rossmann, M. G., McKinlay, M. A., Diana, G. D., Dutko, F. J., Fancher, M., Rueckert, R. R. & Heinz, B. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3304–3308.
23. Richardson, J. S. (1979) *Adv. Protein Chem.* **34,** 167–339.
24. Rossmann, M. G. & Palmenberg, A. C. (1988) *Virology*, in press.
25. Colonno, R. J., Callahan, P. L. & Long, W. J. (1986) *J. Virol.* **57,** 7–12.
26. Mapoles, J. E., Krah, D. L. & Crowell, R. L. (1985) *J. Virol.* **55,** 560–566.
27. Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S. & Alizon, M. (1985) *Cell* **40,** 9–17.
28. Gelderblom, H. R., Hausmann, E. H. S., Özel, M., Pauli, G. & Koch, M. A. (1987) *Virology* **156,** 171–176.
29. Hull, R. (1969) *Adv. Virus Res.* **15,** 365–433.
30. Caspar, D. L. D. & Klug, A. (1962) *Cold Spring Harbor Symp. Quant. Biol.* **27,** 1–24.
31. Coates, A. R. M., Cookson, J., Barton, G. J., Zvelebil, M. J. & Sternberg, M. J. E. (1987) *Nature (London)* **326,** 549–550.