Crystallization of a common cold virus, human rhinovirus 14: "Isomorphism" with poliovirus crystals

(picornavirus structure/x-ray diffraction/reproducibly grown crystals/high-resolution data)

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ABSTRACT Crystals of rhinovirus 14 have been grown reproducibly. They diffract x-rays to a resolution of at least 3.5 Å. The orthorhombic crystal unit cell contains two virions, each situated on a crystallographic twofold axis. At less than 30-Å resolution, the space group approximates to $I222$ with the particles possessing 222 pseudo crystallographic symmetry. The crystals are "isomorphous" with type I polio crystals [Finch, J. T. & Klug, A. (1959) *Nature (London)* 183, 1709-1714; Hogle, J. M. (1982) *J. Mol. Biol.* 160, 663-668], suggesting some similarities of structure between enteroviruses and rhinoviruses.

Human rhinoviruses cause the common cold (1). They are a subgroup, with more than 113 characterized serotypes, of the picornaviruses, a family of viruses that cause a variety of infectious diseases of man and other mammals, including livestock. Although picornaviruses have been subclassified according to antigenic specificity, pH stability, and buoyant density, the architecture, assembly, and molecular biology of these viruses appear to be fundamentally similar (1). To further our understanding of the molecular etiology of picornaviruses in general, and of the common cold virus in particular, we have embarked on a high-resolution structural study of human rhinovirus 14 (R14). Hexagonal crystals of the 1A strain had been reported (2), and these we had earlier examined for their diffraction properties. However, the present crystals of R14 (Fig. 1) diffract to better than 3.5-Å resolution (Fig. 2).

The R14 viruses were grown in rhinovirus-sensitive HeLa cells and purified by a modification of the procedure described for encephalomyocarditis virus (3). Viruses were allowed to attach at room temperature (21°C), usually at a multiplicity of infection of around 10 plaque-forming units per cell. This was followed by an 8- to 10-hr incubation, at 35°C, with agitation. Rhinovirions remained about 90-95% associated with the infected cells, which were recovered by centrifugation (5 min at $800 \times g$). After three cycles of freezing and thawing, followed by centrifugation ($1,000 \times g$) to remove debris, the virus-containing supernatant was supplemented with $MgCl_2$ and deoxyribonuclease I to attain concentrations of 5 mM and 10 mg/ml, respectively. This solution was incubated for 30 min at room temperature, warmed to 37°C (at which time trypsin was added to a final concentration of 0.5 mg/ml), and then incubated for an additional 10 min. One-tenth volume of 10% Sarkosyl along with EDTA to 10 mM was then added to the solution, which was clarified by centrifugation (10,000 rpm, Sorvall SS34 rotor, 10 min). Virus was pelleted by centrifuging in a Beckman type

FIG. 1. R14 crystals in a vapor diffusion well. Crystals are prisms of maximal dimensions $0.6 \times 0.3 \times 0.3$ mm.

30 rotor at 10°C for 4 hr at 27,000 rpm. Pellets were resuspended in 10 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA and 0.1% 2-mercaptoethanol (TEM). The virus was then banded in 7.5-45% sucrose gradients, in TEM buffer, by centrifuging at 10°C for 90 min at 38,000 rpm in a Beckman SW 41 swinging-bucket rotor and collected by using an ISCO density gradient fractionation system. Virus-containing fractions were pooled, diluted with a minimum of 2 vol of TEM, and repelleted by centrifuging in a Beckman Ti 65 rotor at 10°C for 90 min at 48,000 rpm. Virus was resuspended in TEM and stored at 4°C or -70°C prior to use. Yields were 0.5-1.0 mg of virus per 10^9 infected cells, as measured by using $A_{260}^{0.1\%} = 7.7$ (3). Plaque assay tests showed that R14 was 75% neutralized by a 1:10,000 dilution of hyperimmune reference antiserum against R14 (NIH AS no. 2, con 5, 7/25/68) but was not perceptibly neutralized by hyperimmune antiserum against type I poliovirus even at dilutions as low as 1:10.

The R14 suspension was subjected to one further cycle of low- and high-speed centrifugation prior to crystallization. The resulting virus pellets were dissolved in a small volume of 20 mM Tris-HCl buffer, pH 7.5, to give a final concentration of 5-20 mg/ml. This preparation had to be used quickly, because microcrystals often formed during storage and had to be removed by low-speed centrifugation, resulting in a loss of virus.

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Abbreviations: R14, rhinovirus 14; TEM, Tris/EDTA/2-mercaptoethanol.

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FIG. 2. Still photograph taken on an Elliott GX20 rotating anode generator, using a 0.1-mm-wide Cu target. The x-rays were focused with two perpendicular bent mirrors. Crystal-to-film distance was 100 mm. The center of the concentric rings corresponds to the a axis direction. The outermost visible Bragg reflections were from planes with a 3.5-Å spacing.

Crystals were grown at room temperature in vapor diffusion cells that were coated with Dow Corning 4 compound to reduce nucleation and to prevent crystals from adhering to the glass surface of the wells. A solution of $(\text{NH}_4)_2\text{SO}_4$ ($x\%$ saturated) containing 100 mM sodium phosphate buffer at pH 7.2 and 1 mM sodium azide was added to an equal volume of a solution containing R14 virus at y mg/ml (in which $2 < y < 20$ mg/ml) such that the product xy was numerically between 5 and 10 units. The solution was put into a well of the diffusion chamber and equilibrated against $(\text{NH}_4)_2\text{SO}_4$ at around 2.5% saturation. Crystals then grew up to 0.6 mm in length within a few days to a week (Fig. 1).

The R14 crystals were examined with focused CuK_α x-radia-

tion from an Elliott GX20 rotating anode generator, using precession and oscillation cameras. The Laue symmetry was mmm and the lengths of the unit cell edges were $a = 323$, $b = 358$, and $c = 380$ Å (Table 1). The diffraction pattern showed

Table 1. Comparison of R14 and type 1 poliovirus crystals

Virus	Unit cell dimensions, Å			Space group
R14	$a = 323$	$b = 358$	$c = 380$	$P2_12_12$ (pseudo $I222$)*
Type 1 polio	$c = 320$	$a = 353$	$b = 378$	$P2_12_12$ (pseudo $I222$)*

* Permutations of the primitive space group symmetry elements are possible, because the crystal diad has not been identified.

FIG. 3. Screenless precession photographs of the $0kl$ zone ($\mu = 1.5^\circ$) for poliovirus (8) (Left) and the $h0l$ zone ($\mu = 2.0^\circ$) of an R14 crystal (Right). The 323-Å axis is horizontal and the 380-Å axis is vertical.

systematic absences when $h + k + l = 2n + 1$ below 30-Å resolution, although at higher resolution the lattice appeared primitive. Hence, the low-resolution pseudo space group was either $I222$ or $I2_12_12_1$. Assuming a molecular weight of around 8.4×10^6 (1), this gives a volume per molecular mass (V_M) of $5.0 \text{ Å}^3/\text{dalton}$ for $Z = 1$ or of $2.5 \text{ Å}^3/\text{dalton}$ for $Z = 2$, in which Z is the number of particles per unit cell. The value of V_M for viruses is usually in excess of 3.0 (4), but the smallest permissible value of Z is 2 for the $I222$ and 4 for the $I2_12_12_1$ space group. Hence, the only reasonable solution is that the pseudo low-resolution space group is $I222$ with $Z = 2$, the particles being situated on 222 special positions with their icosahedral twofold axes coincident with the crystallographic twofold directions. The shortest distance between particle centers is then 307 Å, consistent with the diameter of other icosahedral viruses of similar molecular weight (cf. ref. 5) and within the range of 230–300 Å of partially hydrated picornaviruses observed in electron microscope studies (6).

The pseudo low-resolution space group provides information on the packing of the two particles in the cell. The only primitive space groups consistent with the arrangement of symmetry elements in the $I222$ group are the three permutations of $P2_12_12_1$. Although in principle it is easy to differentiate between these groups, we were unable to observe convincing axial reflections with odd indices in order to identify the diad direction. The $P2_12_12_1$ space group contains only a single crystallographic diad, which must coincide with a twofold axis of the virus particle. The deviation from the exact body-centered symmetry can arise due to a small translation along or a small rotation about the crystal diads. Both conditions will slightly displace the particle twofold axes from coincidence with the orthogonal crystal pseudo-diads.

The R14 crystals are remarkably similar to crystals formed by the type I Mahoney strain of poliovirus (Table 1) reported by Schwerdt and Schaffer in 1956 (7) and later analyzed in x-ray diffraction experiments by Finch and Klug (8). The crystals possess similar morphologies (8–10), unit cells, and space groups (8). In addition to these resemblances, the intensity distribution on the published 1.5° screenless precession photograph ($0kl$ zone) for poliovirus (8) is markedly similar to that of the corresponding 2° precession photograph ($h0l$ zone) for R14 (Fig. 3). Therefore, the two virus crystals may be considered to be “isomorphous” to at least a 30-Å resolution view of the struc-

tures. (The quotation marks signify that isomorphism is limited to low resolution.)

The “isomorphism” between the R14 and type I poliovirus crystals reflects similarities in structure and crystal packing of the virions. Similarities of internal structure probably represent common features in folding of the protein subunits, which would not be surprising in view of the common patterns in capsid polypeptide composition and assembly (1, 11) indicative of a common ancestor for enteroviruses and rhinoviruses. On the other hand, the common packing mode of particles in the crystals, which is in part determined by the surface of the virions, was unexpected because of the differing antigenicity and receptor specificity of the viruses. The “isomorphism” requires that the surface contacts are not only at similar angles to each other but also at homologous positions on the viral subunit. It is, therefore, a remarkable coincidence or evidence for a previously unsuspected chemical similarity at the contact region of the two types of virus. Evidence for lack of common surface features was primarily the complete absence of crossneutralization by heterologous hyperimmune antisera, a point that has been confirmed in this study. However, neutralizing antibodies do not necessarily define the entire set of antigenic determinants on the surface of the virions, and common surface features might be found by using precipitant procedures.

Picornaviruses of the genus *Enterovirus* and of the genus *Rhinovirus* have many similarities, including gene order, as well as modes of translation, processing, and assembly. However, the rhinoviruses exhibit two features, acid lability and permeability to cesium ions, not shared by enteroviruses. One possible explanation for the difference in permeability is that the protein subunits of R14 are less densely packed than those of poliovirus. Consistent with this explanation is the observation that cesium-free R14 sediments about 4% more slowly than poliovirus (12). If the virion masses were identical, this would correspond to a 4% greater diameter—i.e., 316 Å for R14 compared to 304 Å for poliovirus. However, as reported above, the interparticle distance measured in wet crystals of R14 (307 Å) is indistinguishable from that of poliovirus (304 Å). Given the uncertainty in the mass of R14, it is not clear whether the slower sedimentation behavior of rhinovirus is due to a smaller mass or to some subtler feature of its structure. Clearly, comparison of human rhinovirus 14 and type I poliovirus (13) by high-resolution structural analysis should provide important new insights into the nature of the structural features responsible for the differences between enteroviruses and rhinoviruses. Such comparisons should also help elucidate evolutionary relationships of the viruses and clarify how their surface chemistry determines receptor-dependent tissue specificity and neutralization by antibody.

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