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Unusual Diffraction of Type B Influenza Virus Neuraminidase Crystals

BY MING LUO*

Center for Macromolecular Crystallography and Department of Microbiology, University of Alabama at Birmingham, University Station, Birmingham, Alabama 35294, USA

W. GRAEME LAVER

John Curtin School of Medical Research, Australia National University, Canberra, ACT 2601, Australia

AND GILLIAN AIR

Department of Microbiology, University of Alabama at Birmingham, University Station, Birmingham, Alabama 35294, USA

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Abstract

An unusual X-ray diffraction pattern by tetragonal crystals of a type B influenza virus neuraminidase was observed in that the odd-l reflections were missing or diffuse while the even-l reflections were sharp and strong. A statistical analysis showed that an error (ε) in the spacing of successive planes of neuraminidase molecules was randomly distributed along the c direction, which resulted in such an unusual diffraction pattern. The error ε follows the Bernoullian distribution and may be caused by a flexible loop on the top surface of the neuraminidase.

Introduction

Neuraminidase (NA) is a glycoprotein found on the lipid envelope of the influenza virus. Upon virus attachment to the host cell receptor, the neuraminidase cleaves off terminal sialic acid residues from the polysaccharides of the cellular receptor *via* hydrolysis of the α-ketosidic linkages. This cleavage of sialic acids from its receptor, its virus hemagglutinin and from mucin facilitates transportation of virions from the site of infection and the departure of the mature progeny virions (Burnet & Stone, 1947). The protein has a subunit molecular weight of 50 000 daltons and exists in a tetrameric form both on the viral surface and as free molecules in solution. The enzymatically active heads of NA (missing about 80 amino acids from the N terminus) can be removed from the viral surface by protease cleavage and subsequently purified for crystallization.

The NA crystals

NA from type B influenza virus was crystallized in different crystalline forms according to Bossart, Babu, Cook, Air & Laver (1988), Lin, Luo, Laver, Air, Smith & Webster (1990) and Air, Laver, Luo, Stray, Legrone & Webster (1990). Among them, crystals from four different virus strains are tetragonal, body centered. Form I was grown with NA from the B/Hong-Kong/73 virus by vapor diffusion of hanging drops against 1.9 M potassium phosphate. Crystals appeared as rectangular prisms. X-ray diffraction photographs were taken using radiation from both a Rigaku K200 rotating anode and the Cornell High-Energy Synchrotron Source (CHESS). By precession and cone-axis photography, the space group of form I was determined as $I422$, with unit-cell dimensions $a = b = 123.0$, $c = 165.0$ Å. Form II was grown with

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* To whom correspondence should be addressed.

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NA from the B/mem/89 virus by vapor diffusion of hanging drops against 20% polyethylene glycol (PEG) 3350 in saline (0.15 M NaCl). X-ray diffraction was recorded on a Siemens area detector using X-rays from a Rigaku K200 rotating anode. The space group is also $I422$, with unit-cell dimensions $a = b = 123.7$, $c = 165.9$ Å. The two crystalline forms are essentially identical. Forms III and IV were grown with NA from B/Singapore/79 and B/Oregon/80, respectively, by vapor diffusion of hanging drops against 1.7 M potassium phosphate, but not fully characterized.

X-ray diffraction

Fig. 1 is a 0.3° oscillation photograph taken from a Form I crystal at CHESS. The crystal-to-film distance is 70 mm and the exposure time is 90 s. The X-ray beam is approximately parallel to $\mathbf{c}^*$ and $\mathbf{a}^*$, $\mathbf{b}^*$ are along the diagonal directions. The diffraction presents such an unusual pattern in that reflections are diffuse whenever l is odd and are strong and sharp when l is even. It is obvious in Fig. 1 that reflections with $l = -4, -2, 0, 2, 4, 6, \ldots$ are sharp while reflections with $l = -3, -1, 1, 3, 5, \ldots$ are either diffuse or absent. Some of the diffuse reflections are not on the lattice point. The distribution of the ordered and diffuse reflections are anisotropic. Sharp reflections are seen up to 2.2 Å resolution in the a^* and b^* directions but no sharp or diffuse reflections can be seen beyond 5.5 Å resolution along the c^* direction (Fig. 2). The crystal lattice is completely destroyed beyond 5.5 Å resolution in the c direction. This unusual diffraction pattern is probably caused by disorder of the crystal lattice along the c axis (see below). On photographs taken with rotating-anode radiation, there is only very faint scattering corresponding to odd-l reflections. This is probably due to deterioration of the crystal lattice upon long-time exposure to X-rays (usually 4 h). Forms III and IV showed the same unusual diffraction pattern as form I (data not shown) and they appeared to have the same space group. However, no such defects were observed in the diffraction by the form II crystals when diffraction data were collected on the area detector.

Statistical analysis of the disorder

There is no apparent lattice disorder along $\mathbf{a}$ and $\mathbf{b}$ of the form I crystals as shown by the strong and sharp reflections in the a^* and b^* directions when l is even. The disorder is only seen in the c direction as indicated by the diffuse reflections when l is odd. Therefore, it may be considered that the molecules of NA tetramers are packed orderly in ab planes and the ab planes are stacked perpendicular to c with certain errors in spacing. The spacing in the a and b directions must be fairly precise since the resolution reaches 2.2 Å along $\mathbf{a}^*$ and $\mathbf{b}^*$ when l is even. However, the spacing between the ab planes is erroneous in the c direction throughout the crystal. Similar disorder, although not as dramatic, was ob-

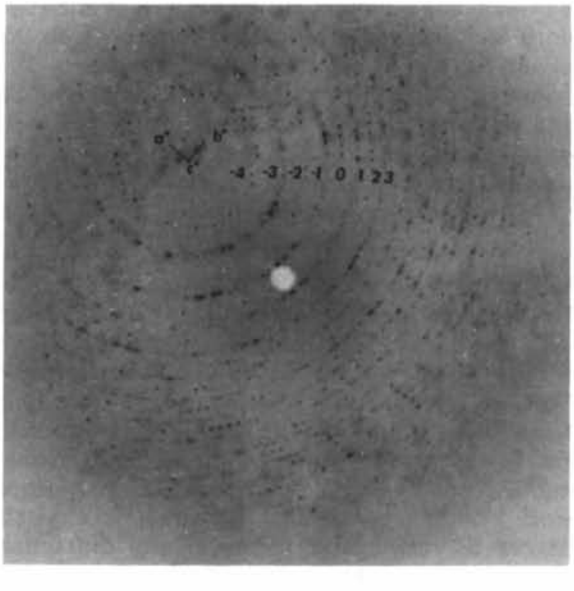


Fig. 1. Diffraction pattern from a form I crystal taken by oscillation photography at CHESS. The oscillation angle about the spindle axis is 0.3° and the exposure time is 90 s. The wavelength of the synchrotron X-rays is 1.56 Å and the crystal-to-film distance is 70 mm. The diffraction photograph was taken with the X-ray beam roughly parallel to $\mathbf{c}^*$.

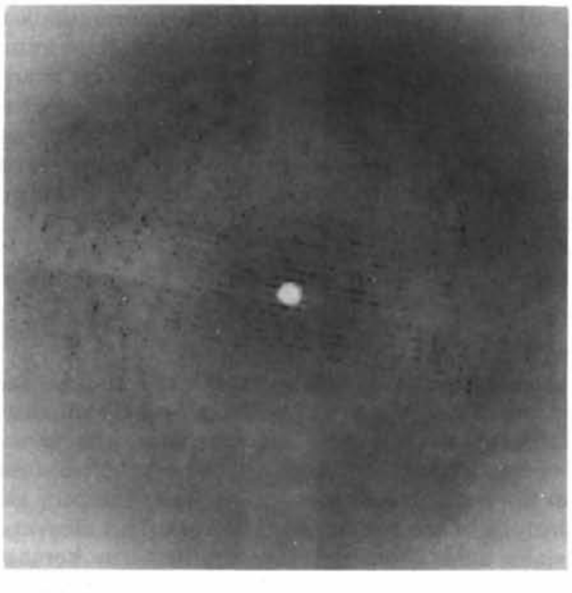


Fig. 2. The second oscillation photograph taken after rotating the crystal about the spindle axis by 90° relative to the photograph shown in Fig. 1. The other conditions are identical to those of Fig. 1. The c^* axis is roughly perpendicular to the X-ray beam.

served in imidazole methaemoglobin (Howells & Perutz, 1954; Bragg & Howells, 1954; Cochran & Howells, 1954) and horse erythrocyte catalase (Glauser & Rossmann, 1966) crystals. The analysis of the anisotropic distribution of the disordered reflections by the above authors led to the understanding of the erroneous packing in the crystal lattice and its statistical feature. We have statistically analyzed the disorder of the form I crystals of NA.

The NA tetramer has dimensions of 100 × 100 × 60 Å as shown by electron microscopy (Laver, Luo, Bossart, Babu, Smith, Accavitti, Tulloch & Air, 1989) and the crystal structure of type A NA (Varghese, Laver & Colman, 1983). There are large flexible-loop structures on the upper surface of the tetramer. It is reasonable to assume that the disorder of the crystal lattice was caused by the flexible loops on the upper surface, which should make up half of the molecular contact of the NA tetramers along **c**. The other half is made up by the lower surface, which does not cause any disorder. Let ε be the average error in cell length along **c** between successive *ab* planes consisting of two layers of NA tetramers interacting with each other *via* lower surface contact (Fig. 3). Molecules are arranged orderly in *ab* planes with a space group of *P*422. These *ab* planes exist only in theory because the NA tetramers are not in contact in the crystal-lattice plane. The ordered *ab* planes are stacked along **c** according to *I*422 space-group symmetry with a random displacement of $\pm\varepsilon$ between successive *ab* planes. The origin of the $(n+1)$th *ab* planes are shifted by $(\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$. Thus the lattice has statistically a space group of *I*422, but the unit-cell length along **c** is not precise. If a zero *ab* plane is chosen to contain the origin of the three-dimensional lattice, the scattering by an atom in the *n*th *ab* plane, equivalent to the atom (x_j, y_j, z_j) in the zero *ab* plane, can be written as

$$s(h, k, l) = f_i \exp(2\pi i\{hx_j + ky_j + l[z_j + n/2 + (n-2m)\varepsilon]\}), \tag{1}$$

where *m* is an integer between 0 and *n*. One *ab* plane is positioned at **c**/2. The $(n-2m)\varepsilon$ term represents the displacement error of the *n*th *ab* plane in spacing along **c** and the error has a value between $-n\varepsilon$ and $n\varepsilon$. Since the errors only occur in the *c* direction, we need only treat the *l* direction as a non-periodic lattice. The random displacement errors along **c** follow the Bernoullian distribution as shown by Cochran & Howells (1954) and the probability of finding the equivalent atom at a given position with error $(n-2m)\varepsilon$ in *c* is given as $\{\mathbf{C}_{n-m}(\frac{1}{2})^n\}$. By summation over the total planes (N) in the crystal, the structure factor will be given by

$$\mathbf{F}(h, k, l) = \sum_i \sum_n \sum_m \mathbf{C}_{n-m}(\tfrac{1}{2})^n f_i \exp(2\pi i\{hx_j + ky_j + l[z_j + n/2 + (n-2m)\varepsilon]\}). \tag{2}$$

Equation (2) can be rewritten as

$$\mathbf{F}(h, k, l) = \mathbf{E}(h, k, l)\mathbf{F}(h, k, l)_{\text{normal}}, \tag{3}$$

where

$$\mathbf{E}(h, k, l) = \sum_n \sum_m \mathrm{C}_{n-m}(\tfrac{1}{2})^n \times \exp\{2\pi i l[n/2 + (n-2m)\varepsilon]\}. \tag{4}$$

$\mathbf{E}(h, k, l)$ is the multiplier caused by the displacement error in *c* and $\mathbf{F}(h, k, l)_{\text{normal}}$ stands for the normal terms of the structure factor.

We shall now calculate the multiplier $E(h, k, l)$. If (4) is summed over *m*, we shall have, according to the binomial theorem,

$$\begin{aligned} E(h, k, l) &= \sum_n (\tfrac{1}{2})^n \exp(\pi i n l) \exp(2\pi i n l\varepsilon) \\ &\quad \times [1 + \exp(-4\pi i l\varepsilon)]^n \\ &= \sum_n (\cos 2\pi l\varepsilon)^n \exp(\pi i n l). \end{aligned} \tag{5}$$

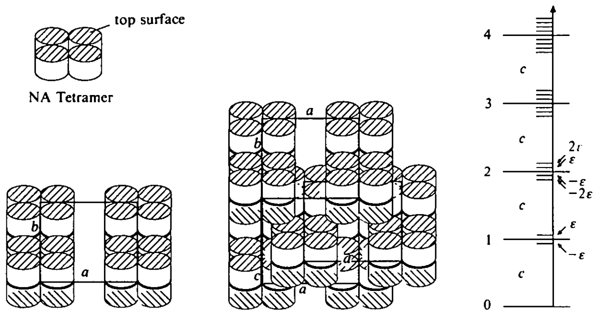
The sum of (5) over *n* can be written as

$$E(h, k, l) = [1 - \cos(2\pi l\varepsilon)^N \exp(\pi i N l)] \times [1 - \cos(2\pi l\varepsilon) \exp(\pi i l)]^{-1}. \tag{6}$$

If ε is very small (close to zero), $\cos(2\pi l\varepsilon)$ will be close to 1 and (6) may be written as

$$E(h, k, l) = \frac{\exp(-\pi i l/2)}{\exp(-\pi i N l/2)} \frac{\sin(\pi N l/2)}{\sin(\pi l/2)}. \tag{7}$$

Since *N* can be considered to approach infinity, $E(h, k, l)$ is close to zero unless *l* is an even integer.



ab plane Packing of *ab* planes Distribution of ε

Fig. 3. A diagram showing the arrangements of the NA tetramers in the *ab* plane and the stacking of the *ab* planes along **c**. The tetramer is represented by four cylinders of which the upper surface is marked with shaded areas. The tetramers are packed in the *ab* plane following *P*422 symmetry. The fourfold axis of the tetramer coincides with the crystallographic fourfold axis and the two layers of NA molecules within an *ab* plane make contact with each other through the lower surface. When the *ab* planes are stacked along **c**, the contacts between successive *ab* planes are through the upper surface of the NA tetramer, on which the flexible loops of the structure are located. Errors (ε) between the *ab* planes are distributed randomly as indicated on the right.

In the case of the form I crystals, ε indeed seems to be very small because the reflections were seen up to 5.5 Å resolution along **c**. For the assumption that $\cos(2\pi l\varepsilon)$ is close to 1 and $l_{\max}$ is $(165\ \text{Å})/(5.5\ \text{Å}) = 30$ to hold, the value of ε for any two successive *ab* planes cannot be larger than 0.04 (or $0.04 \times 165 = 7$ Å) for $0.99 \leq \cos(2\pi l\varepsilon)$. Because of this statistical property of the random displacement errors in c spacing, normal reflections were observed only when l is even. According to formula (7), odd-l reflections should not be present at all. However, the odd-l reflections did not disappear completely. Some diffuse scattering was seen at positions of the odd-l reflections. This might be because the crystal has limited dimensions and/or the distribution of the displacement errors is not truly binomial. The crystals might be constructed by stacking of ordered microcrystals consisting of several *ab* planes along **c**, instead of randomly distributed single *ab* planes. Nevertheless, the statistical analysis demonstrated that the random distribution of a small error in the spacing of the *ab* planes in the **c** direction will cause such an unusual diffraction pattern in which the odd-l reflections would be missing.

Amino acid substitutions in the loops

The crystals of B/mem/89 (form II) have the same space group as the other three crystalline forms, but they do not exhibit the disorder along **c**. The conditions used for crystallizing B/mem/89 NA did not produce crystals for the other three strains. Amino acid sequence analysis of the NAs indicated that there are five residues in the B/mem/89 NA head which are different from all the other strains (Air, Laver, Luo, Stray, Legrone & Webster, 1990). These differences are Glu148 → Lys148, Glu250 → Lys250, Asp340 → Asn340, Arg345 → Leu345 and Lys436 → Glu436. These residues are all located on the upper surface of the NA in comparison with the NA structure of the type A influenza virus and the substitutions all involved charge changes. It will be interesting to see what structural changes cause the unusual disorder when the atomic structure of the type B NA is determined.

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The Piezomagnetoelectric Effect

By Hans Grimmer

Laboratorium für Materialwissenschaften, Paul Scherrer Institut, CH-5232 Villigen PSI, Switzerland

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Abstract

Taking time reversal into account there are 122 crystallographic Shubnikov point groups and 21 limiting Shubnikov point groups containing ∞-fold rotation axes. The restrictions on the form of the tensor describing the piezomagnetoelectric effect are given for all these groups and compared with results of other authors. Antiferromagnetic materials with noncentrosymmetric point groups containing space-time inversion are suitable candidates for measuring the piezomagnetoelectric effect.

1. Introduction

The piezomagnetoelectric effect was first considered by Rado (1962) who derived its form for materials