CRYSTALLIZED N-TERMINAL DOMAIN OF INFLUENZA VIRUS MATRIX PROTEIN M1 AND METHOD OF DETERMINING AND USING SAME

Inventors: Ming Luo; Bingdong Sha, both of Birmingham, Ala.

Assignee: University of Alabama Research Foundation, Birmingham, Ala.

Filed: Aug. 1, 1997

Related U.S. Application Data

Provisional application No. 60/023,564, Aug. 7, 1996.

International Patent Classification

C12N 7/00; C12N 7/04; C07K 14/11; C12Q 11/70

Field of Search

530/1350; 435/236; 235/1; 435/1350; 378/174; 378/173; 424/1204.1; 424/1206.1

References Cited

PUBLICATIONS


Pub...
FIG. 1b

The diagram shows the Xmerge $|f_1-f_2|$ for tmp.df. The graph plots the absolute difference $|\Delta|$ against resolution (sin(theta/lambda)).

- Solid line with triangles: all 4715 reflections
- Dashed line with circles: 3866 acentric reflections
- Solid line with squares: 849 centric reflections

The y-axis ranges from 0 to 96.7, and the x-axis ranges from 0.0167 to 0.135.
Xmerge Merging R for tmp.df

Resolution (sin(theta/lambda))

FIG. 1c
FIG. 2a

|Delta|

- all 3671 reflections
- 2929 acentric reflections
- 742 centric reflections

Resolution (sin(theta/lambda))

0.0141 0.0382 0.0623 0.0864 0.111 0.135
Xmerge - Merging R for tmp.df

<table>
<thead>
<tr>
<th>Deltal</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.294</td>
<td></td>
</tr>
<tr>
<td>0.235</td>
<td></td>
</tr>
<tr>
<td>0.176</td>
<td></td>
</tr>
<tr>
<td>0.118</td>
<td></td>
</tr>
<tr>
<td>0.0588</td>
<td></td>
</tr>
</tbody>
</table>

- all 3671 reflections
- 2929 acentric reflections
- 742 centric reflections

FIG. 2b

Resolution (\(\sin(\theta)/\lambda\))
<table>
<thead>
<tr>
<th>Res. limits (Å)</th>
<th>I/sigma (I)</th>
<th>Rsymm %</th>
<th>% of observations</th>
<th>Observations/ reflections</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.00</td>
<td>3.78</td>
<td>44.1</td>
<td>2.7</td>
<td>95.4</td>
</tr>
<tr>
<td>3.78</td>
<td>3.00</td>
<td>42.4</td>
<td>3.9</td>
<td>96.0</td>
</tr>
<tr>
<td>3.00</td>
<td>2.62</td>
<td>33.0</td>
<td>5.4</td>
<td>95.9</td>
</tr>
<tr>
<td>2.62</td>
<td>2.38</td>
<td>24.6</td>
<td>7.8</td>
<td>96.0</td>
</tr>
<tr>
<td>2.38</td>
<td>2.21</td>
<td>18.5</td>
<td>10.2</td>
<td>95.9</td>
</tr>
<tr>
<td>2.21</td>
<td>2.08</td>
<td>9.1</td>
<td>17.5</td>
<td>88.6</td>
</tr>
<tr>
<td>All</td>
<td>33.7</td>
<td>4.6</td>
<td>94.7</td>
<td></td>
</tr>
</tbody>
</table>

Space group: P3_{1}2_1, a=b=67.17Å, c=135.30Å

FIG. 3
CRystallized N-Terminal Domain of Influenza Virus Matrix Protein M1 and Method of Determining and Using Same

BACKGROUND OF THE INVENTION

This application claims benefit, pursuant to 35 U.S.C. § 119, of applicants' provisional U.S. Ser. No. 60/023,564, filed Aug. 7, 1996, the contents of which are hereby incorporated by reference. This application was supported, in part, by a grant from NASA (NAGW-819).

FIELD OF THE INVENTION

The present invention relates to the field of crystallography and, particularly, to the characterization of the structure of matrix protein M1 of influenza virus neuraminidase and, specifically, the determination of the crystal structure of the N-terminal domain of M1, the domain itself, the crystallized domain, methods of purifying and crystallographically determining the three-dimensional structure of that domain, and methods of using the crystal structure of N-terminal domain of M1 to design pharmaceuticals.

BACKGROUND OF THE INVENTION

Influenza virus is an enveloped virus which contains eight separate segments of negative-stranded RNA genome. There are two spike glycoproteins on the surface of the viral membrane envelope, the receptor binding hemagglutinin (HA), and the neuraminidase (NA). The core ribonucleoprotein (RNP) encapsulated in the viral membrane envelope is composed of an RNA polymerase and RNA-binding nucleoproteins (NP) (1). The interaction of RNPs with the membrane is mediated by the matrix protein M1 (252 amino acids, Mr=27 kd), which are tightly associated with the RNP cores while interacting with the cytoplasmic tails of the spike glycoprotein and the viral membrane (2). Through the binding of hydrophobic domains to the virion lipid envelope, M1 maintains the structural integrity of the virus particle (3,4). On the other hand, the interaction of M1 with RNP cores is mostly electrostatic at neutral pH (5,6). The M1 can easily be dissociated from the RNP cores by low pH treatment (7,8).

In addition to packaging the RNP cores during virion assembly, M1 also directs the transportation of RNPs into or out of the nucleus (9,10). Upon entry of the virus into the new host cell, M1 is dissociated from RNPs as the result of reducing pH in the fusion endosome, allowing the RNPs to enter the nucleus. When progeny viral RNPs are produced, newly synthesized M1 escort the RNPs out of nucleus and target them to the assembly site on the cellular membrane where HA and NA are located. It is therefore highly desirable to provide a method of deducing the crystal structure of M1 and of providing a method of using this structure to provide antiviral candidates and M1 inhibitors.

SUMMARY OF THE INVENTION

The present invention provides a crystallized N-terminal domain of M1, wherein the N-terminal domain of M1 is crystallized such that the three dimensional structure of the crystallized N-terminal domain of M1 can be determined to a resolution of 2.1 Å or better, and wherein the three-dimensional structure of the uncrystallized N-terminal domain of M1 cannot be determined to a resolution of 2.1 Å or better.
antiviral compound can be screened for having improved binding to M1.

In yet another embodiment, the present invention provides a purified N-terminal domain of M1. In a further embodiment, the present invention provides this purified N-terminal domain of M1, comprising the amino acid sequence of SEQ. ID. NO. 1 (see below). In one embodiment, “N-terminal domain” means the amino acid sequence of SEQ. ID. NO. 1. One of skill in the art would recognize that various amino acid substitutions could be made to this polypeptide. Such modifications, so long as they are the basic and novel utility of the present invention is not disturbed, are understood to be within the scope of the present invention. For instance, a polypeptide could be constructed containing the first 150 amino acids, from position 1 (perhaps by cleavage of some of the amino acids and addition at the N-terminal end). Such a polypeptide is intended to be within the scope of the present invention. To the extent that the crystal structures of such analogous polypeptides are similar to the structure set forth herein, such structures are included within the scope of the present invention.

In yet another embodiment, the present invention provides a method for designing a candidate compound for screening for improved binding to or inhibition of influenza virus M1, comprising evaluating the three-dimensional structure of the crystallized N-terminal domain of M1, and synthesizing a candidate binding compound based on this structure. Moreover, the present invention provides for the use of the three-dimensional crystal structure of the crystallized N-terminal domain of M1 for improved binding to M1.

In a further embodiment, the present invention provides the three-dimensional crystal structure of influenza virus protein M1 as set forth elsewhere herein. In a further embodiment, the present invention provides a crystallized polypeptide having three-dimensional crystal structure.

In yet another embodiment, the invention provides a method for designing a candidate compound for screening as an antiviral for the prevention or treatment of influenza virus infection, comprising evaluating the three-dimensional crystal structure set forth herein, and synthesizing a candidate compound based on this structure. Moreover, the present invention provides for the use of the three-dimensional crystal structure as set forth herein for screening candidate compounds for inhibition of influenza virus M1.

In a further embodiment, the present invention also provides for the use of the N-terminal domain of M1 for screening candidate compounds for inhibition of influenza virus M1.

Finally, the present invention provides a crystallized N-terminal domain of M1.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1a shows the Harker section (Z=1/3) of the difference Pattern of Os derivative of M1 crystals.

FIG. 1b shows the structure factor amplitude difference between native and Os derivative data sets versus resolution. From top to bottom, the curves are for all reflections, acentric reflections and centric reflections, respectively.

FIG. 1c shows the merging R-factor between native and Os derivative data sets versus resolution. From top to bottom, the curves are for all reflections, acentric reflections and centric reflections, respectively.

FIG. 2a shows a section of solvent flattened map (FOM=0.89) at 5 Å resolution.

FIG. 2b shows the merging R-factor between native and Pt derivative data sets versus resolution. From top to bottom, the curves are for all reflections, acentric reflections and centric reflections, respectively.

FIG. 2c shows a section of solvent flattened map (FOM=0.89) at 5 Å resolution.

FIG. 2d shows a slice of electron density map at 3.5 Å resolution corresponding to an alpha-helix.

FIG. 3 shows atomic coordinates of the three dimensional crystal structure of influenza virus protein M1.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Figures.

Before the present methods and structures are disclosed and described, it is to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. It must be noted that, as used in the specification and appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

Throughout this application, where publications are referenced, the disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. Publications have been referenced herein by placing the number in parentheses. These publications are listed according to their respective numbers in the “Reference” section hereinbelow.

The present invention therefore provides a crystallized N-terminal domain of M1, wherein the N-terminal domain of M1 is crystallized such that the three dimensional structure of the crystallized N-terminal domain of M1 can be determined to a resolution of 2.1 Å or better, and wherein the three dimensional structure of the uncrystallized N-terminal domain of M1 cannot be determined to a resolution of 2.1 Å or better.

In a further embodiment, the present invention provides a method of extracting the M1 protein of influenza virus comprising stripping the membrane proteins from influenza virus, removing the stripped membrane proteins, thereby leaving the M1-RNP complex, and releasing M1 from the M1-RNP complex by suspending the complex in a solution comprising NaH2PO4, Benazamide, and NaN3. In a preferred embodiment, the solution has a pH of from about 3 to about 5, preferably about 4.0. In a further embodiment, this method is extended to include purifying the released M1.

In yet another embodiment, the present invention provides a method of extracting the N-terminal domain of the M1 protein of influenza virus comprising stripping the membrane proteins from influenza virus, removing the stripped membrane proteins, thereby leaving the M1-RNP complex, and releasing M1 from the M1-RNP complex by suspending the complex in a solution comprising NaH2PO4, Benazamide, and NaN3. In a preferred embodiment, the solution has a pH of from about 3 to about 5, preferably about 4.0. In a further embodiment, this method further comprises crystallizing the 18 kd polypeptide corresponding to the N-terminal domain of M1.
of M1 to a resolution of less than about 2.1 Å, wherein the N-terminal domain of M1 is present at a concentration of about 3 to about 20 mg/ml, more preferably from about 4 to about 10 mg/ml and most preferably about 5 mg/ml, and the crystallization takes place at from 4 to 32º C, more preferably from 10 to 26º C, even more preferably at about 16 to about 24º C, and even more preferably 20º C, to thereby obtain crystals of space group P3_21 or P3_121. The crystals may have an approximate a = 68.0 Å and approximate c = 136.57 Å. The crystallization, in one embodiment, may occur using hanging drops and the vapor diffusion method. Alternatively, other crystallization methods may be used. For instance, a temperature variation may be used to produce crystals, or crystallization in space may be used to improve resolution. The crystallization, in another embodiment, may occur over 20% PEG 3350. For instance, organic chemicals (e.g., isopropanol), inorganic chemicals (e.g., (NH_4)_2SO_4, NaH_2PO_4), and other molecular weight PEG (from MPD to PEG20,000) may be used.

In yet another embodiment, the present invention provides a method for determining the three dimensional structure of the crystallized N-terminal domain of M1 to a resolution of less than about 2.1 Å, wherein the N-terminal domain of M1 is present at a concentration of about 3 to about 20 mg/ml, more preferably from about 4 to about 10 mg/ml, even more preferably about 5 mg/ml and the crystallization takes place at from about 4 to about 32º C, more preferably from 10 to 26º C, even more preferably at about 16 to about 24º C, and even more preferably 20º C, to thereby obtain crystals of space group P3_21 or P3_121, and then analyzing the N-terminal domain of M1 to determine the three dimensional structure of the crystallized N-terminal domain of M1. A preferred embodiment, the invention provides that purified N-terminal domain of M1 comprises the amino acid sequence of SEQ. ID. NO. 1.

In yet another embodiment, the present invention provides a purified N-terminal domain of M1. In a further embodiment, the invention provides that purified N-terminal domain of M1, comprising the amino acid sequence of SEQ. ID. NO. 1, wherein the N-terminal domain of M1 is present at a concentration of about 3 to about 20 mg/ml, more preferably from about 4 to about 10 mg/ml, even more preferably about 5 mg/ml and the crystallization takes place at from about 4 to about 32º C, more preferably from 10 to 26º C, even more preferably at about 16 to about 24º C, and even more preferably 20º C, to thereby obtain crystals of space group P3_21 or P3_121, and then analyzing the three dimensional structure of the crystallized N-terminal domain of M1, wherein the antiviral compound can be screened for having improved binding to M1. In a further embodiment, the antiviral compound is a peptide or polypeptide. The crystallization, in one embodiment, may occur using hanging drops and the vapor diffusion method. Alternatively, other crystallization methods may be used. For instance, a temperature variation may be used to produce crystals, or crystallization in space may be used to improve resolution.

In yet another embodiment, the present invention provides a method for using a purified N-terminal domain of M1. In a further embodiment, the present invention provides a method for designing an antiviral compound for the prevention or treatment of influenza virus infection, comprising evaluating the three dimensional structure of the crystallized N-terminal domain of M1 produced by crystallizing a purified N-terminal domain of M1 to a resolution of less than about 2.1 Å, wherein the N-terminal domain of M1 is present at a concentration of about 3 to about 20 mg/ml, more preferably from about 4 to about 10 mg/ml, even more preferably about 5 mg/ml and the crystallization takes place at from about 4 to about 32º C, more preferably from 10 to 26º C, even more preferably at about 16 to about 24º C, and even more preferably 20º C, to thereby obtain crystals of space group P3_21 or P3_121, and synthesizing an antiviral compound based on the three-dimensional crystal structure of the crystallized N-terminal domain of M1, wherein the antiviral compound can be screened for having improved binding to M1. In a further embodiment, the antiviral compound is a peptide or polypeptide. The crystallization, in one embodiment, may occur using hanging drops and the vapor diffusion method. Alternatively, other crystallization methods may be used. For instance, a temperature variation may be used to produce crystals, or crystallization in space may be used to improve resolution.

In yet another embodiment, the present invention provides a method for screening an improved binding or inhibition of influenza virus M1, comprising the amino acid sequence of SEQ. ID. NO. 1, and synthesizing a candidate binding compound based on the three-dimensional crystal structure of the crystallized N-terminal domain of M1, wherein the candidate binding compound can be screened for having improved binding to M1. In a further embodiment, the candidate compound is a peptide or polypeptide. The crystallization, in one embodiment, may occur using hanging drops and the vapor diffusion method. Alternatively, other crystallization methods may be used. For instance, a temperature variation may be used to produce crystals, or crystallization in space may be used to improve resolution.

In yet another embodiment, the present invention provides a method for improving binding or inhibition of influenza virus M1, comprising the amino acid sequence of SEQ. ID. NO. 1, and synthesizing a candidate binding compound based on the three-dimensional crystal structure of the crystallized N-terminal domain of M1, wherein the candidate binding compound can be screened for having improved binding to M1. In a further embodiment, the candidate compound is a peptide or polypeptide. The crystallization, in one embodiment, may occur using hanging drops and the vapor diffusion method. Alternatively, other crystallization methods may be used. For instance, a temperature variation may be used to produce crystals, or crystallization in space may be used to improve resolution.

In yet another embodiment, the present invention provides a method for using a purified N-terminal domain of M1. In a further embodiment, the present invention provides a method for designing an antiviral compound for the prevention or treatment of influenza virus infection, comprising evaluating the three dimensional structure of the crystallized N-terminal domain of M1 produced by crystallizing a purified N-terminal domain of M1 to a resolution of less than about 2.1 Å, wherein the N-terminal domain of M1 is present at a concentration of about 3 to about 20 mg/ml, more preferably from about 4 to about 10 mg/ml, even more preferably about 5 mg/ml and the crystallization takes place at from about 4 to about 32º C, more preferably from 10 to 26º C, even more preferably at about 16 to about 24º C, and even more preferably 20º C, to thereby obtain crystals of space group P3_21 or P3_121, and synthesizing an antiviral compound based on the three-dimensional crystal structure of the crystallized N-terminal domain of M1, wherein the antiviral compound can be screened for having improved binding to M1. In a further embodiment, the antiviral compound is a peptide or polypeptide. The crystallization, in one embodiment, may occur using hanging drops and the vapor diffusion method. Alternatively, other crystallization methods may be used. For instance, a temperature variation may be used to produce crystals, or crystallization in space may be used to improve resolution.
In yet another embodiment, the present invention provides a crystallized polypeptide having the three-dimensional crystal structure set forth in the previous table. The present invention also provides a method for designing candidate compounds based on the three-dimensional crystal structure. These compounds are chemically synthesized and their biological activity is assayed. For those compounds which show activity, they are associated or complexed with the crystal for further X-ray diffraction analysis to map the interactions of the compound with the crystal structure.

From the resulting inhibitor-target crystal structure, one of ordinary skill in the art could construct further improved candidate compounds. The steps set forth in the preceding paragraph are repeated and refined as desired.

With this in mind, examples using the preferred embodiments of the above-described methods and structures are set forth hereinafter. Other features of the invention will become apparent from the following examples, which are for illustrative purposes only and are not intended as a limitation upon the present invention.

EXAMPLE I

Virus Preparation

Since M1 constitutes 40% of the total protein in the influenza virus, intact virions were used as the source for purification of the M1 protein. This also ensures that the M1 protein under study is authentic. Attempts to express the purification of the M1 protein. This also ensures that the M1 protein was centrifuged at 8,000 rpm in a Beckman JA10 rotor for three hours at 4°C. The virus band was collected from the upper middle area, and was pelleted again in a Beckman SW28 rotor at 25K rpm for three hours at 4°C. Finally, the virus was resuspended in Ca**Mg**-Saline solution for storage.

EXAMPLE II

Protein Extraction and Purification

In order to strip off the lipid membrane and the membrane embedded surface proteins, 1 M of the purified virus preparation was loaded on a three-step sucrose gradient (8). The gradient consisted of, from bottom to top, 3 M 32% sucrose and 5 M 17% sucrose in 0.15 M NaCl, 10 mM Hepes (pH 7.2) containing 1% of nonionic detergent NP40, and 2 M 10% sucrose in 0.15 M NaCl, 10 mM Hepes (pH 7.2) without detergent. After centrifugation in a Beckman SW41 rotor at 21 K rpm for three hours at 4°C, the M1-RNP complex was pelleted to the bottom of the centrifuge tube whereas the membrane associated proteins remained in the gradient solution. The pellet of the M1-RNP complex was resuspended in 1 M of 50 mM NaH2PO4, 50 mM, 5 mM Benzamidine and 0.02% NaN3 (pH of from 3.0 to 5.0, preferably 4.0) to release the M1 protein from the M1-RNP complex (8). The RNP cores (lacking M1) were removed by centrifugation in a Beckman SW55 rotor at 22K rpm for one hour at 4°C.

The M1 protein was further purified by gel filtration in a column of Superdex 75 (Pharmacia) mounted on a Pharmacia FPLC system. The M1 protein was pooled about 62 minutes after sample injection at a flow rate of 1 M/min. Compared with the molecular weights of the protein standards, the apparent molecular weight of the eluted M1 protein was about 50 kd. Because the molecular weight of M1 monomer derived from its amino acid sequence was expected to be 27 kd (II), the M1 protein appears to form a dimer in solution at acidic pH.

However, the M1 protein was not stable when concentrated to 5 mg/ml prior to crystallization. A stable fragment of 18 Kd was identified after two week storage of the concentrated protein sample at room temperature. Dot-blotting showed that this major fragment of the M1 protein was still recognized by rabbit anti-M1 polyclonal antibodies. The molecular weight of the fragment was determined by Mass Spectrum (PE Sciex API III) to be 18,230 dalton. The M1 fragment remained soluble after transfer of the concentrated protein into a buffer with pH 7.2. The soluble fragment, at pH 7.2, was then incubated with M1-free RNP cores obtained during M1 purification. The mixture was pelleted and analyzed by SDS-PAGE gel. The gel showed that, similar to the native M1 protein, the M1 fragment was still able to bind RNP cores at neutral pH.

Since this fragment can still bind to RNP cores, M1 can be conceptualized as a two-domain protein. The C-terminal domain binds the RNP cores through hydrophilic interactions which can be interrupted by reducing the pH. The N-terminal domain binds the membrane. Without wishing to be bound by theory, the N-terminal domain probably has hydrophobic properties because the intact M1 protein aggregates at neutral pH while the C-terminal domain binds the RNP cores through hydrophilic interactions which can be interrupted by reducing the pH. The N-terminal end thus binds the membrane. The concept of a two domain protein is consistent with the function of M1. During virus production, M1 is synthesized and transported to the nucleus as a dimer where it binds to RNP cores by the C-terminal domain. This is consistent with the RNA binding and anti-transcriptase activity data which were mapped by
monoclonal antibodies to be within the amino acid sequence at position 90 to position 164. The association of M1 with RNP cores signals, perhaps through a conformational change of M1, the transportation of the M1-RNP complex to the assembly site on the cellular membrane. Only the RNP-associated M1 N-terminal domain can bind to the membrane and the C-terminal tails of the spike glycoproteins, HA and NAA. This completes the function of M1 during virion assembly. During virion entry, the C-terminal domain of M1 is dissociated from the RNP cores due to the low pH of the fusion endosome while the N-terminal domain remains associated with the membrane and the spike glycoprotein tails by hydrophobic interactions. The dissociation of M1 releases naked RNP cores and uncovers the signal for nucleus targeting carried by RNP with RNP cores signals, perhaps through a conformational change induced by low pH. Unlike the matrix protein of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV), which are single domain proteins forming a trimer, the M1 protein is a two domain protein functioning as a dimer. The membrane anchoring of M1 occurs through an entirely separate N-terminal protein domain, rather than a covalent myristoyl modification, as found in HIV and SIV matrix proteins.

**EXEMPLARY III**

**Crystallization, Data Collection and Processing**

Crystallization was carried out in hanging drops by the vapor diffusion method. However, one of skill in the art would recognize that other crystallization methods may be used. For instance, a temperature variation method may be used to produce crystals, while crystallization in outer space may be used to improve resolution. Nonetheless, for the hanging drop method, the protein concentration was about 5 mg/ml. Large crystals (0.05 mm x 0.05 mm x 0.3 mm) of the 18 kDa M1 protein fragment could be grown at 20°C over 20% PEG 3350 in two months. X-ray diffraction data were collected at Brookhaven National Laboratory on beamline X-12C. 60 frames from two crystals were collected and the data were processed by the HKL package. The crystals were classified as belonging to space group P321 or P3121 with a = b = 68.74 Å, c = 136.57 Å. The data were about 56% complete to 2.35 Å resolution and the Rsymm was 0.11 when reflections with I>2σ(I) were included in the data. Completeness 96.2%, Rsymm 4.2%, mean Rmerge 0.77. Phasing power 1.19, Position 577, 385, 439. A native data set (to 2.1 Å resolution) was collected at NSLS under cryo conditions and the statistics are shown in Table 1.

**TABLE 1**

The Native Data from NSLS Synchrotron

<table>
<thead>
<tr>
<th>Res. limits (Å)</th>
<th>Ωs (l)</th>
<th>Rsymm (%)</th>
<th>% of observations</th>
<th>% of reflections</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.00</td>
<td>3.78</td>
<td>44.1</td>
<td>2.7</td>
<td>95.4</td>
</tr>
<tr>
<td>3.78</td>
<td>3.00</td>
<td>42.4</td>
<td>3.9</td>
<td>96.0</td>
</tr>
<tr>
<td>3.00</td>
<td>2.62</td>
<td>33.0</td>
<td>5.4</td>
<td>95.9</td>
</tr>
<tr>
<td>2.62</td>
<td>2.58</td>
<td>24.6</td>
<td>7.8</td>
<td>96.0</td>
</tr>
<tr>
<td>2.38</td>
<td>2.21</td>
<td>18.5</td>
<td>10.2</td>
<td>95.9</td>
</tr>
<tr>
<td>2.21</td>
<td>2.08</td>
<td>9.11</td>
<td>17.5</td>
<td>89.6</td>
</tr>
<tr>
<td>All</td>
<td>33.7</td>
<td>4.6</td>
<td>94.7</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Space group: P321, a = b = 67.17 Å, c = 135.30 Å

Because there are no reported homologous structures to M1, the structural solution relies on the conventional multiple isomorphous replacement method. Three data sets were collected under identical conditions from a SIEMENS Highstar multiwire detector, mounted on a Rigaku rotating anode source with the Oxford cryo system. These three data sets are a new native and two derivative data sets. The statistics are shown in Table 2.

**TABLE 2**

Refinement of heavy Atom Parameters

<table>
<thead>
<tr>
<th>Native KPOCl</th>
<th>K2AOHFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>3.5 Å</td>
</tr>
<tr>
<td>Completeness</td>
<td>96.2%</td>
</tr>
<tr>
<td>Rsymm</td>
<td>4.2%</td>
</tr>
<tr>
<td>Rmerge</td>
<td>0.77</td>
</tr>
<tr>
<td>Phasing power</td>
<td>1.19</td>
</tr>
<tr>
<td>Position</td>
<td>577, 385, 439</td>
</tr>
</tbody>
</table>

The Harker section of the difference patterns of the Os and Pt derivatives are shown in FIGS. 1a, 1b, 1c, 1d, 2a, 2b, 2c and 2d. The amplitude difference and merging R factors versus resolution for the two derivatives are shown also shown in FIGS. 1a, 1b, 1c, 1d, 2a, 2b, 2c and 2d. Finally, a section of solvent flattened map (FOM=0.89) at 5 Å resolution is shown in FIG. 2c, and a region corresponding to an α-helix at 3.5 Å is shown in FIG. 2d.

**REFERENCES**

The following references are incorporated in their entirety herein by this reference:


Sequence Listing

SEQ. ID. NO. 1 represents amino acids 2–164 of the N-terminal domain of influenza virus matrix protein M1:

SEQ. ID. NO.1: SLLTEVETYVLSIIPSGPLKAE...

It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

What is claimed is:
1. A crystallized N-terminal domain of the M1 protein of influenza virus comprising the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:1, wherein the N-terminal domain of M1 is crystallized to obtain crystals of space group P321 or P3121 with approximate a=68.0 Å and approximate c=136.5 Å such that the three dimensional structure of the crystallized N-terminal domain of M1 can be determined to a resolution of about 2.1 Å or better.

Sequence Listing

<160> NUMBER OF SEQ ID NOS: 1
<210> SEQ ID NO 1
<211> LENGTH: 163
<212> TYPE: PRT
<213> ORGANISM: Influenza Virus
<400> SEQUENCE: 1
Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Ile Pro Ser
Gly Pro Leu Lys Ala Glu Ala Gln Arg Leu Glu Val Phe Ala
Gly Lys Asn Thr Asp Leu Glu Val Met Glu Trp Leu Lys Thr Arg
Pro Ile Leu Ser Pro Leu Thr Lys Ile Leu Gly Phe Val Phe Thr
Leu Thr Val Pro Ser Glu Arg Gly Leu Glu Lys Arg Arg Phe Val Glu
Asn Ala Leu Ala Gly Asn Gly Asp Pro Pro Ala Met Asp Lys Ala Val
Lys Leu Tyr Arg Lys Leu Asp Arg Glu Ile Thr Phe His Gly Ala Lys
Glue Ile Ser Leu Ser Tyr Ser Ala Gly Leu Ala Ala Cys Met Gly
Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Glu Val Ala Phe Gly
Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Arg Ser
His Arg Gln

a) stripping the membrane proteins from influenza virus;
b) removing the stripped membrane proteins, thereby leaving the M1-RNP complex;
c) releasing M1 from the M1-RNP complex by suspending the complex in a solution comprising NaH2PO4, Benzaamidine, and NaN3;
d) purifying the released M1;
e) concentrating the purified M1 to a concentration of from 3 mg/ml to 20 mg/ml; and
f) after a period of time sufficient for the formation of an 18 kd protein fragment corresponding to the N-terminal domain of M1, collecting the 18 kd polypeptide corresponding to the N-terminal domain of M1.

4. The method of claim 3, wherein the time period is from 7 to 21 days.

5. The method of claim 3, further comprising crystallizing the 18 kd polypeptide corresponding to the N-terminal domain of M1 to a resolution of less than about 2.1 Å, wherein the N-terminal domain of M1 is present at a concentration of from 3 to 20 mg/ml and the crystallization takes place at from 4 to 32° C., to thereby obtain crystals of space group P321 or P3,21.

6. The method of claim 3, wherein the pH of the solution of step c) is from 3.0 to 5.0.

7. The method of claim 5, wherein the crystallization occurs in hanging drops using the vapor diffusion method.

8. A method for determining the three dimensional structure of the crystallized N-terminal domain of the M1 protein of influenza virus comprising the amino acid sequence set forth in the Sequence Listing as SEQ ID NO:1 to a resolution of about 2.1 Å, wherein the N-terminal domain of M1 is present at a concentration of from 3 to 20 mg/ml and the crystallization takes place at from 4 to 32° C., to thereby obtain crystals of space group P321 or P3,21.

9. The method of claim 8, wherein the analyzing is by x-ray diffraction.

10. The method of claim 8, wherein the crystallization occurs in hanging drops using the vapor diffusion method.

11. The crystallized N-terminal domain of M1 produced by the process of claim 5.

12. A method for designing a potential antiviral compound for the prevention or treatment of influenza virus infection, comprising:
a) obtaining crystals of the N-terminal domain of M1, wherein the crystals have the space group P3,21 or P3,21 with approximate a=68.0 Å and approximate c=136.57 Å such that the three dimensional structure of the crystallized N-terminal domain of M1 can be determined to a resolution of about 2.1 Å or better,
b) evaluating the three dimensional structure of the crystallized N-terminal domain of M1;
c) synthesizing the potential antiviral compound based on the three-dimensional crystal structure of the crystallized N-terminal domain of M1;
d) contacting an influenza virus or an influenza viral protein with the potential antiviral compound; and
e) assaying the influenza virus for infectivity or monitoring the influenza viral protein for activity, or both, whereby a decrease in the infectivity of the influenza virus or a change in the activity of the influenza viral protein indicates the compound may be used for the prevention or treatment of influenza virus infection.

13. The method of claim 12, wherein the antiviral compound is a peptide or polypeptide.

14. The method of claim 12, wherein the crystallization occurs in hanging drops using the vapor diffusion method.

15. The method of claim 12, wherein the crystal has the three dimensional crystal structure of influenza virus protein M1 as set forth in FIG. 3.

16. The method of claim 12, wherein the influenza viral protein comprises M1.

17. A method for designing a candidate compound for screening for binding to or inhibition of influenza virus M1, comprising:
a) utilizing the three dimensional structure of a crystallized N-terminal domain of M1 which is defined by the atomic coordinates in FIG. 3; and
b) designing a candidate binding compound based on the three-dimensional crystal structure of the crystallized N-terminal domain of M1 for binding to M1.

18. The method of claim 17, wherein the candidate compound is a peptide or polypeptide.

* * * * *