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Structure determination of hemagglutinin-neuraminidase in complex with drugs

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1 Introduction

The influenza virus represents one of the most alarming viruses spreading worldwide. In 2009, the H1N1 subtype caught the world by surprise when it manifested itself as the pandemic of the new century, infecting more than 61 million humans, with more than 12,450 deaths. The influenza virus is an RNA virus that uses two glycoproteins, hemagglutinin (HA) and sialidase/neuraminidase (NA) to assist in its infection of avian and mammalian cells. NA in particular, by hydrolyzing sialic acids from sialoglycoconjugates at the surface of the host cell, facilitates the release of newly formed virions. Pharmaceutical companies used NA as a target for drug design, due to the fact that its inhibition prevents release of progeny virions. One additional characteristic of NA is that, although it may differ in its amino acid sequence when comparing influenza strains, all NAs among species share one nearly identical active site's architecture. Therefore, drugs targeted to the NA of one virus strain will most likely act on other strains as well, making of this enzyme a perfect target for influenza virus inhibition and treatment.

Only a few reports concerning the mammalian NAs have been published so far. Characterized on the basis of their subcellular localization and substrate specificities, four distinct mammalian sialidases were identified: cytosolic, lysosomal, plasma membrane bound, and associated with mitochondria. In the past few years, we have solved and reported the first three-dimensional structure of the human cytosolic sialidase Neu2 in the apo and inhibitor bound forms [1]. We then extended this work to some functional and structural analysis of the human enzyme in complex with influenza virus targeted drugs, and showed that some side effects occurring while treating the influenza virus with NA-targeted drugs might be resulting from specific inhibitions of human NAs [2]. Finally, we attempted to decipher the human sialidase enzymatic mechanism by chemical trapping of various reaction intermediates [3]. In the present work, we are pushing further structure-based drug-design studies and screen a larger number of potential inhibitors for parainfluenza hemagglutinin-neuraminidases to check their interaction with the non-human neuraminidases first, and hopefully identify a molecule that would specifically target the virus NAs without affecting the human enzymes.

2 Experiment

Macromolecular crystallography diffraction experiments were performed at the beam line AR-NW12A. The x-ray wavelength was set to 1.0000 Å. X-ray intensities were recorded on an ADSC Q210r CCD x-ray detector. The sample exchange robot PAM [4] was extensively used for facilitating semi high-throughput screening of various inhibitors complexed to the enzyme.

3 Results and Discussion

Complete data sets were collected for various potential inhibitors, to resolutions up to 2 Å. Structure determination was performed based on our unpublished apo-form structure, and confirmed the presence of inhibitor molecules inside the active site of the enzyme. Fig.1 shows the electron density for one of the inhibitor inside the active site of the enzyme, clearly identified.

These results represent a milestone in the developments of new drugs against the parainfluenza virus hemagglutinin-neuraminidase. The complex structures will need to be tested for human neuraminidase inhibition, and will eventually open the window to more developments for even more specific recognition of the viral enzyme.

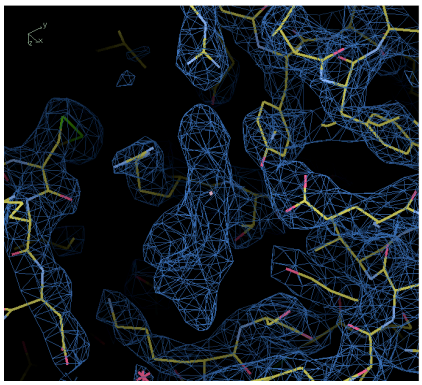

Fig. 1: electron density inside the parainfluenza hemagglutinin-neuraminidase active site

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References

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