BL-17A/2011G542; BL-1A/2010G705; BL-17A/2011G234

Insight into structural diversity of influenza virus hemagglutinin

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1 Introduction

The influenza virus surface protein, HA, is a prime target of the host immune response. HA0 is cleaved by cellular proteases into HA1 and HA2, a fusion-competent form and undergoes conformational changes at low pH [1]. We have solved the crystal structure of recombinant HA protein from a 2009pdm isolate, A/Korea/01/2009 (KR01) and complex structure of KR01 HA with the Fab fragment of a neutralizing monoclonal antibody GC0757 that binds to various H1 subtype strains. We observed a significant conformational difference between KR01 HA and other HAs derived from other influenza virus isolates, A/Thailand/CU44/2006 (CU44), A/Brisbane/59/2007 (BR59) and A/Gyeongnam/684/2006 (Gy684), including CA04, and DA01.

2 Experiments

KR01, CU44, BR59, and Gy684 HAs were produced in insect cells using recombinant baculovirus expression vectors. Active form of HA was purified by Ni-NTA, Mono Q ion-exchange and Superdex 200HR size exclusion chromatography. Crystals of KR01, CU44, BR59, and Gy684 HAs were screened by the hanging drop vapor diffusion method. Small single crystals of KR01 and CU44 HAs were obtained in 100 mM HEPES (pH 7.5), 20% PEG 3350, and 0.2 M NaCl at 4°C, and in 100 mM Tris-HCl (pH 7.0), 22% PEG, 0.2 M calcium acetate at 24°C, respectively, that diffracted to sufficiently high resolution. In the case of the complex of KR01 HA and Fab0757, 1 µl of the complex (10 mg/ml) was mixed with 1 µl of screening solution of 20% PEG3350 and 200 mM potassium iodide and incubated at 4°C. Diffraction data were collected with the crystals flash-cooled at 100 K in a stream of liquid N_2 in the mother liquor containing 22% glycerol using synchrotron radiation sources. The microcrystals of KR01 HA, CU44 HA and KR01 HA-Fab0757 diffracted to 2.7 Å, 2.5 Å and 2.8 Å resolutions, respectively, at beamlines BL-1A and BL-17A at Photon factory (Tsukuba, Japan). All data were processed and scaled using the HKL2000 program. The crystal structures of KR01 HA, CU44 HA and KR01 HA-Fab0757 were solved by molecular replacement [CCP4 or PHENIX]. Manual adjustment of the backbone and side chains was conducted in Coot. Crystallographic refinement was carried out using the program refmac5.

3 Results and Discussion

The crystal structure of KR01 HA revealed a V-shaped head-to-head arrangement, which is not seen in other HA proteins including CU44 HA. The KR01 HA-Fab0757 complex structure also exhibited a head-to-head arrangement of HA (Fig. 1). Both native and Fab complex structures reveal different spatial orientation of HA1 relative to HA2, indicating that HA is flexible and dynamic at neutral pH (Fig. 2). Our structures provide important insight into conformational flexibility of HA that may have a significant impact on diverse conformations of HA1 over HA2.

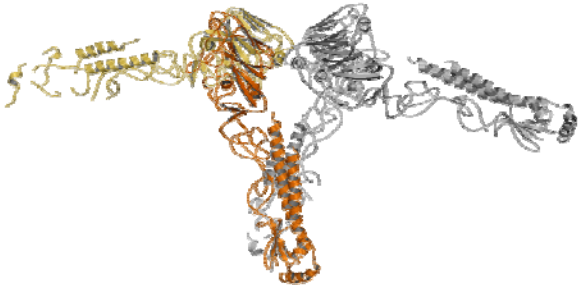

Fig. 1. Structure of KR01 HA. Head-to-head arrangement of KR01 HA in the asymmetric unit of the crystal (orange and yellow) and symmetry-related molecules (grey).

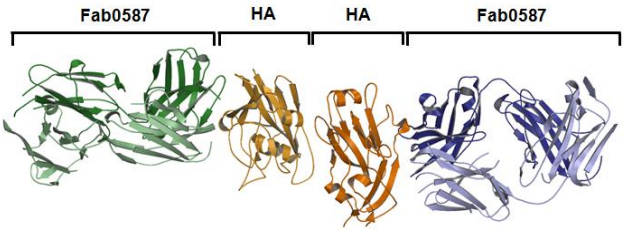

Fig. 2**.** Overall structure of KR01 HA-Fab0757 complex. Head regions of KR01 HA are colored in orange and light brown, heavy chains are blue and lime green, and light chains are light blue and aqua marine.

We postulate that KR01 HA on the virus surface adopts flexible conformations with substantially low energy barrier, as a loosely assembled trimer. It will be interesting to see whether the interactions of HA with adjacent HA molecules would have a direct effect on generating unusual conformations that we observe in this study. We targeted several amino acid residues that are located at the monomer-monomer interface as well as HA1-HA2 interface and mutation experiments are currently underway.

Acknowledgement

This work was supported by grants from Mid-career Researcher Program through NRF funded by the MEST (2010-0029242) and from Transgovernmental Enterprise for Pandemic Influenza in Korea (TEPIK, 2011-A103001).

References

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