Binding of sulfatide to recombinant hemagglutinin of influenza A virus produced by a baculovirus protein expression system

Tadanobu Takahashi[2], Hiroaki Satoh[3], Masahiro Takaguchi[2], Suguru Takafuji[3], Hisashi Yokoyama[3], Satoshi Fujii[3], and Takashi Suzuki[1,2]

[1]Global COE Program, [2]Department of Biochemistry, [3]Department of Physical Biochemistry, Graduate School of Pharmaceutical Sciences, University of Shizuoka

Sulfatide is abundantly expressed in mammalian organs, including the intestine and trachea, in which influenza A viruses (IAVs) replicate. Sulfatide binds to IAV particles and inhibits the viral sialidase activity under low-pH conditions [1,2]. Sulfatide is synthesized by two transferases, ceramide galactosyltransferase (CGT) and cerebroside sulfotransferase (CST), and is degraded by arylsulfatase A (ASA). In previous study, we demonstrated that association of sulfatide with hemagglutinin (HA) delivered to the cell surface induces translocation of the newly synthesized IAV ribonucleoprotein (vRNP) complexes from the nucleus to cytoplasm, by using genetically produced cells in which sulfatide expression was down-regulated by RNA interference against CST mRNA or overexpression of ASA gene and in which sulfatide expression was up-regulated by overexpression of both CST and CGT genes. In IAV-infected cells, nuclear export of vRNP complexes and IAV replication were inhibited by addition of an anti-HA monoclonal antibody (MAb) or anti-sulfatide MAb, both of which inhibited IAV binding to sulfatide. Similarly, in animal models, anti-sulfatide MAb protected mice against lethal challenge with pathogenic influenza A/WSN/33 (H1N1) virus. These findings suggested that association of newly synthesized HA to sulfatide on the cell surface was an initial signal for increasing nuclear export of vRNP and that inhibitors of HA binding to sulfatide could be useful as novel anti-IAV agents [3]. However, it is not known whether there is direct binding of HA to sulfatide. In this study, we found that recombinant HA, which was produced by a baculovirus protein expression system from the HA gene of duck/HK/313/4/78 (H5N3), bound to sulfatide in a dose-dependent manner and that the binding was inhibited by a specific antibody [4]. Our results indicate that the recombinant HA is useful for elucidation of the binding domain of HA with sulfatide and for the development of new anti-IAV agents.

[1] T. Suzuki et al., Biochem. J. 318, 389-393 (1996)

[2] T. Suzuki et al., FEBS Lett. 553, 355-359 (2003)

[3] T. Takahashi et al., J. Virol. 82, 5940-5950 (2008)

[4] T. Takahashi et al., J. Biochem. 147, 459-462 (2010)