Molecular Modeling of Catalytic and Lectin Domains in Neuraminidase A from *Streptococcus pneumoniae*

Yana Sharapova, Dmitry Suplatov, Vytas Švedas

Lomonosov Moscow State University, Belozersky Institute of Physicochemical Biology and Faculty of Bioengineering and Bioinformatics, Vorobjev hills 1-40, Moscow 119991, Russia,
vytas@belozersky.msu.ru

Sialidases NanA, NanB, and NanC of a pathogenic bacteria *Streptococcus pneumoniae* are involved in host colonization and considered as potential drug targets [1]. These homologous enzymes are remarkably different in amino acid sequences and domain organization, have diverse substrate preferences, and roles in pathogenesis. PDB currently deposits coordinates separately for the catalytic and lectin domains of NanA [2, 3]. At the same time the two-domain structures of NanB and NanC are available in which the catalytic and lectin domains form a large interdomain contact surface [4, 5]. NanA additionally contains a membrane anchor domain which attaches the protein to a bacterial cell wall. However the structure of this domain is available neither for NanA, nor for any other homologous protein. Molecular modeling has been used to build the two-domain structure of the catalytic and lectin domains of NanA based on template structures of NanB and NanC. It was shown that corresponding domains are structurally similar among homologs but the interdomain linker in NanA is much longer compared to NanB and NanC (25 amino acids versus 9 and 10 amino acids). Molecular dynamics simulations of the obtained two-domain NanA model showed that the catalytic and lectin domains of NanA start moving away from each other after the first 30 ns and the two domains spatially separate during the 200-500 ns while being covalently connected by the interdomain linker. In contrast to that the interactions between catalytic and lectin domains of NanB and NanC remain stable under the same conditions. We have further investigated the domain-domain contact surfaces of different pneumococcal sialidases. Visual structural analysis coupled with APBS approach to calculate electrostatic properties of the protein surfaces showed that interface between the catalytic and lectin domains in NanB and NanC is enforced by a number of polar and ionic interactions and contains a hydrophobic core whereas homologous areas of NanA contain different amino acid residues which do not

support interaction between the two domains. Protein-protein docking of catalytic and lectin domains was able to correctly predict the interdomain interface in NanB and NanC but was not able to find a domain-domain contact surface in NanA. To conclude, molecular modeling has shown that catalytic and lectin domains of NanA connected by a long linker do not form a stable interdomain interface and are spatially separated in a solution. The phenomenon of spatially separated domains in protein structures has been previously discussed in the literature and is considered as evolutionary adaptation to increase the proteins` operational efficiency [6]. In case of NanA, which is secreted in the extracellular space, a more mobile lectin domain can capture the host receptor [7] and thus facilitate its interaction with a less mobile catalytic domain.

This work was supported by the Russian Science Foundation grant #15-14-00069. The supercomputer complex of the Lomonosov Moscow State University was used for resource-intensive computations [8].

1. Kadioglu, A., Weiser, J. N., Paton, J. C., Andrew, P. W. (2008). The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. *Nat. Rev. Microbiol.*, **6(4)**: 288-301.
2. Gut, H., Xu, G., Taylor, G. L., Walsh, M. A. (2011). Structural basis for Streptococcus pneumoniae NanA inhibition by influenza antivirals zanamivir and oseltamivir carboxylate. *J. Mol. Biol.*, **409(4)**: 496-503.
3. Yang, L., Connaris, H., Potter, J. A., Taylor, G. L. (2015). Structural characterization of the carbohydrate-binding module of NanA sialidase, a pneumococcal virulence factor. *BMC Struct. Biol.*, **15(1)**: 15.
4. Gut, H., King, S. J., Walsh, M. A. (2008). Structural and functional studies of Streptococcus pneumoniae neuraminidase B: An intramolecular trans-sialidase. *FEBS Lett.*, **582(23-24)**: 3348-3352.
5. Owen, C. D., Lukacik, P., Potter, J. A., Sleator, O., Taylor, G. L., Walsh, M. A.

(2015). Streptococcus pneumoniae NanC structural insights into the specificity and mechanism of a sialidase that produces a sialidase inhibitor. *J. Biol. Chem.*, **290(46)**: 27736-27748.

6. Ma, B., Tsai, C. J., Haliloğlu, T., Nussinov, R. (2011). Dynamic allostery: linkers are not merely flexible. *Structure,* **19(7)**: 907-917.
7. Thobhani, S., Ember, B., Siriwardena, A., Boons, G. J. (2003). Multivalency and the mode of action of bacterial sialidases. *J. Am. Chem. Soc*., **125(24)**: 7154-7155.
8. Voevodin, V. V., Zhumatiy, S. A., Sobolev, S. I., Antonov, A. S., Bryzgalov, P. A., Nikitenko, D. A., Stefanov K. S., Voevodin, V. V. (2012). Practice of "Lomonosov" supercomputer. *Open Systems J*, **7:** 36-39.