O-Linked glycosylation in Acanthamoeba polyphaga mimivirus

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Abstract: Acanthamoeba polyphaga mimivirus is a member of the giant nucleocytoplasmic large DNA viruses, infecting various Acanthamoeba spp. The genomes of giant viruses encode components previously thought to be exclusive to cellular life, such as proteins involved in nucleic acid and protein synthesis. Recent work on enzymes involved in carbohydrate biosynthesis and metabolism show that instead of utilizing host cell resources, Mimivirus produces its own glycosylation machinery. To obtain a more detailed view of glycosylation in Mimivirus, we developed a periodate oxidation-based method to selectively enrich Mimivirus surface glycoproteins. O-Glycosylation inMimivirus glycoproteins was identified by permethylation and matrix-assisted laser desorption/ionization-mass spectrometry analyses of beta-eliminated glycans.We sequenced 26 previously undescribed O-glycans, most of which contain glucose as their reducing end saccharide. These data will facilitate future studies on the functional significance of glycosylation in Mimivirus.

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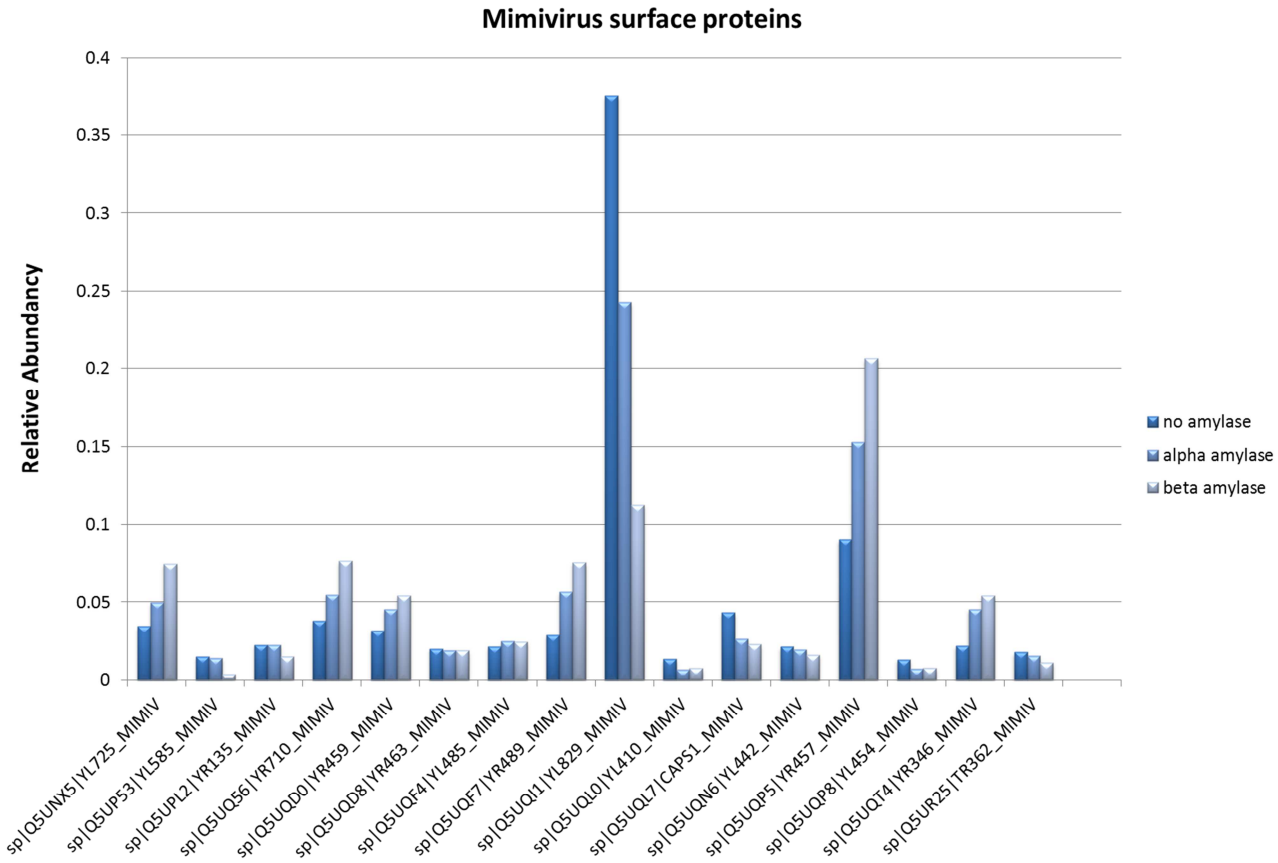
Supplemental Figure 2



Mimivirus particles were digested with alpha-amylase, beta-amylase or untreated prior biotinylation with sulpho-NHS-biotin. Biotinylated proteins were extracted and purified via an avidin-cartridge. The eluates were digested with trypsin and subjected to mass spectrometric peptide sequencing. Mimivirus protein abundance indices extracted from Mascot search results (emPAI values, (1)) were normalized for each LC-MS run and the values for 16 most abundant Mimivirus proteins are plotted. An increase in relative abundance after amylase digestion is a result of increased solvent accessibility of amine functional groups to the biotin probe. This indicates the presence of glycosylation on that particular protein or glycosylation from a neighboring protein in the immediate vicinity on the virus surface.

Supplemental reference

1. Ishihama, Y., Oda, Y., Tabata, T., Sato, T., Nagasu, T., Rappsilber, J., and Mann, M. (2005) Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Mol Cell Proteomics* 4, 1265-1272