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H1N1 2009 pandemic influenza virus: Kinetic, structural and thermodynamic analysis of the H275Y, I223V and S247N neuraminidase resistant mutants

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Influenza is an acute viral infection that can cause serious complications and death, especially among elderly individuals and patients at risk. Neuraminidase, which plays an essential role in virus replication, is the main influenza drug target. At present, two neuraminidase inhibitors (NAIs) are licensed worldwide for therapeutic and prophylactic uses (oseltamivir marketed as Tamiflu and zanamivir, Relenza) and two others have been authorized in various countries for the emergency treatment during pandemics. However, drug resistant viruses readily emerge because of the high mutation rate of their RNA dependent RNA polymerase. Indeed, resistance to oseltamivir, the most prescribed NAI was detected not only during treatment and prophylaxis but also in influenza virus variants in untreated individuals. Novel neuraminidase inhibitor resistance substitutions I223V and S247N alone or in combination with a major oseltamivir resistance mutation H275Y have been observed recently in the 2009 pandemic H1N1 viruses. We overexpressed the ectodomain of the wild type neuraminidase from the influenza virus A/California/07/2009 (H1N1) as well as recombinants containing H275Y, I223V and S247N single mutation and the H275Y, I223V and H275Y, S247N double mutants in Drosophila Schneider S2 cells and purified them by one-step purification using a streptavidin derivative. In order to quantify the level of resistance we enzymologically characterized these enzymes with the set of in-house designed and synthesized derivatives of oseltamivir. Thermodynamic analyses of oseltamivir binding to neuraminidase monomutants were performed by protein micro-calorimetry. Finally, we crystallized neuraminidase variants in complexes with oseltamivir to structurally explain the resistance mechanism.

Biography

Jana Pokorna has completed her PhD in Biochemistry from Charles University in Prague in 2013. She is working as a Postdoctoral Fellow at the Institute of Organic Chemistry and Biochemistry ASCR, v.v.i. Her research interests are activity, inhibition, drug and resistance development focusing on HIV protease and neuraminidase from the influenza virus. She has published 11 peer viewed papers and she is the author of 3 patents.

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