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Kinetic, thermodynamic and structural analysis of tamiphospor binding to neuraminidase of H1N1 (2009) pandemic influenza

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The influenza virus causes a severe infection that is responsible for up to half a million deaths each year. The only class of drugs that have been successful targets the viral glycoprotein neuraminidase. Two inhibitors have been approved (Tamiflu, Relenza) and two others have been authorized in various countries for the emergency treatment during pandemics. However, the ongoing development of resistance towards approved inhibitors together with efficient transmission of resistant viruses among humans represents a serious threat to public health. The approved influenza neuraminidase inhibitors are designed to have (oxa) cyclohexene scaffolds to mimic the oxonium transition state during enzymatic cleavage of sialic acid. Their active forms contain the carboxylate that interacts with three arginine residues in the active site of neuraminidase. Recently, the phosphonate group was successfully used as an isostere of carboxylate in oseltamivir and this compound, tamiphosphor, was discovered as a highly active neuraminidase inhibitor. However, the structure of the complex of this promising inhibitor with neuraminidase has not been solved yet and therefore the binding mode into the active site and comparison with oseltamivir carboxylate binding were proposed by molecular docking experiments. We over expressed the ectodomain of pandemic neuraminidase (residues 82 to 469) from the influenza virus A/California/07/2009 (H1N1) in *Drosophila Schneider* S2 cells. The over expressed neuraminidase with N-terminal tag secreted to the medium was subsequently purified by one-step purification using a streptavidin derivative. The enzyme was first characterized enzymologically by fluorimetric assay using 2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (4-MUNANA) as a substrate and the inhibition constants for oseltamivir carboxylate and tamiphosphor were determined, respectively. The binding of the oseltamivir carboxylate or tamiphosphor to the catalytic domain of neuraminidase was thermodynamically characterized by protein microcalorimetry. To our knowledge, this is the first calorimetric determination of the binding mode of an approved neuraminidase inhibitor. Finally, the complex of the neuraminidase ectodomain with tamiphosphor was successfully crystallized; its structure was determined at 1.72 Å resolution and compared with previously reported oseltamivir carboxylate – neuraminidase complex. In conclusion, we analyzed enzymologically, thermodynamically and structurally tamiphospor binding to an actual pandemic neuraminidase of H1N1 influenza. The biochemical characterization of tamiphosphor as promising neuraminidase inhibitor could help in structure-based design of more potent neuraminidase inhibitors active against major resistant strains.

Biography

Kožíšek Milan is a senior scientist at the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague in the group of Jan Konvalinka, PhD. He finished his PhD in 2010 after defense of his thesis "Overcoming drug resistence: The discovery, design and characterization of new nonpeptidic inhibitors of HIV-1 protease". He is the author of 28 papers in peer-reviewed international journals and 4 patents.

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