Abstract

Influenza virus is responsible for seasonal epidemics among human population. Flu illness usually causes only mild symptoms and can be overcome by few days rest. However, this illness might have fatal consequences for young, elderly and immunocompromised individuals. Some viruses are able to „jump“ across species resulting in rise of new types of virus that can have pandemic potential. The search for new treatment options to prevent emergence of new pandemics is thus a high priority task.

Influenza virus neuraminidase is a protein located on the surface of viral particles. It has enzyme activity and catalyzes release of newly formed viral particles from cytoplasmic membrane of infected host cells. This step is crucial in virus life cycle, so the active site of this enzyme has become an important target for development of anti-influenza drugs.

The neuraminidase inhibitors are currently the only effective drugs used for influenza treatment. Nowadays, there are two drugs against flu used worldwide: Tamiflu (oseltamivir) and Relenza (zanamivir).

Recently, resistant influenza strains have been detected with increasing frequency. Point mutations in the active site of neuraminidase are causing decreased susceptibility of virus to inhibitors. Formerly, it was thought that these mutations would significantly impair viral transmission effectivity and fitness. However, these primary mutations are often accompanied by secondary mutations that compensate for negative effect of the primary mutations to viral fitness.

In this bachelor thesis, the characterization of binding of oseltamivir carboxylate and tamiphosphor was performed with recombinant neuraminidase carrying mutation of serine to arginine at position 247 of recent pandemic virus. This mutation plays significant role in emergence of new influenza strains resistant to commonly used inhibitors. The enzyme kinetic, microcalorimetric and X-ray structural analyses of neuraminidase with inhibitors provide valuable information about the effect of this mutation in inhibitor binding.

Keywords: neuraminidase, enzyme kinetics, crystallography, isothermal titration calorimetry, recombinant proteins

[In Czech]