Abstract

Influenza virus causes severe respiratory infections in birds and mammals and it is responsible for up to half a million deaths of human beings worldwide each year. Two molecular targets in influenza viral life cycle, neuraminidase and M2 proton channel are exploited in treatment. However, the recent emergence of new pandemic type along with increasing resistance against approved drugs has urged the need for a new drug target discovery and potential search of its inhibitor. Recently, an interesting protein-protein interaction between two subunits PA and PB1 of influenza A viral polymerase has been identified by X-ray crystallography as a new promising drug target. The fact that relatively few residues drive the binding and that the binding interface is highly conserved presents an intriguing possibility to identify antiviral lead compounds effective against all subtypes of influenza A virus.

In our laboratory, we expressed and purified two fusion tag constructs of the recombinant C-terminal domain of polymerase acidic subunit (CPA) from the pandemic isolate A/California/07/2009 H1N1. First, GST-CPA fusion protein was used for kinetic evaluation of PA-PB1 interaction by surface plasmon resonance. Moreover, this construct was used in the development of high-throughput screening method for search of interaction disrupting molecules based on AlphaScreen technology. We utilized this assay to determine the effect of truncating minimal PB1 peptide responsible for mediating CPA-PB1 interaction. Furthermore, we designed additional construct of CPA fused with protein His_6-SUMO for efficient over-expression and subsequent crystallization experiments. We set up co-crystallization experiments by the vapor-diffusion method and were later able to obtain 3D structure disclosing information employable in rational drug design. Lastly, the secondary high-throughput screening assay is being developed in order to screen broad compound libraries for the search of novel inhibitors.

Keywords: influenza polymerase, protein-protein interaction, high-throughput screening assay, protein crystallography, AlphaScreen assay, DNA-linked inhibitor antibody assay