Expression of viral proteases

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Several viral diseases cannot be controlled by vaccines, which is why there is a great demand to develop efficient drugs and therapies against viruses, such as human hepatitis C virus (HCV) and human herpesvirus-6 (HHV-6).

Viral proteases are enzymes that have been proven to be essential for the maturation of the virus particles. Thus, these enzymes are needed for the production of infectious particles and the inhibition of proteases could prevent the progression of the infection caused by the virus.

To develop specific inhibitors, which could be used as antiviral agents to both the HCV and HHV-6 proteases, the 3-dimensional structures of the HHV-6 protease and the HCV protease-helicase complex need to be determined by X-ray crystallography.

The HCV NS3 protease has been studied extensively, but new drug target sites are needed. NS3 protein is a bifunctional protein having both helicase and protease activities. The structure determination of the helicase and protease complex together with NS4A protease cofactor could yield information needed for inhibitor drug design. In this study, the DNA encoding helicase gene and the bacterial pET vector containing the DNA encoding the protease and NS4A genes were isolated using polymerase chain reaction (PCR). Attempts were made to ligate the PCR-products together, but more work needs to be done to obtain this.

To contribute towards obtaining the crystal structure of HHV-6 protease, affinity Histidine tags for the N- and C-terminus of the protease were designed to aid in the purification of the protease, which has proven difficult. A PCR-product containing the His-tags at the C-terminus was obtained, and this product will be subject to further expression experiments.

An efficient expression system though for both proteases in a bacterial pET vector has been confirmed.

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