Obtaining Highly Purified 3a NS5A Protein of Hepatitis C Virus

Kim, Woojoo (School: Wayland High School)

Hepatitis C virus (HCV) annually infects 2-3% of the world’s population. The infection causes numerous liver complications, but there is currently no vaccine against HCV. Even the antiviral medications are less effective against specific HCV genotypes, such as genotype 3a. This resistance is due to one of its nonstructural proteins, NS5A, responsible for HCV proliferation and interferon response inhibition. Studying NS5A and its mechanism of action is necessary for effective therapeutic development. Therefore, efficient production of NS5A protein is needed. Here, I produced and purified NS5A by generating a Bacmid with the NS5A gene and transfecting the construct into Sf9 insect cells. Three constructs of amino acids 1-191, 1-198, 1-202 were designed to obtain well-folded protein. The Bacmid was formed in DH10Bac cells by ligating each construct with p42 vector after restriction digestion. The Bacmid was then amplified and transfected in Sf9 cells for the formation of baculovirus. The infection was confirmed by western blot analysis. Transfection was performed up to P3 for higher protein titer due to amplification. Finally, the protein in P3 lysate was collected using the Ni-NTA resin system and was purified after wash and elution with imidazole and DDM. Among the three constructs, two Bacmid samples with 1-198 were confirmed by PCR and were used for protein expression. The western blot analysis confirmed 25 kDa of NS5A protein in 1-198 P3 transfection lysate. The protein was collected from 50 ml of P3 lysate and was purified, resulting in 90% purity and 1 mg/L yield. This method of efficiently producing NS5A protein lays the foundation for future studies that aim to create NS5A inhibitors and direct-acting antivirals for the treatment of hepatitis C disease.

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