CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

Application Note

Synthesis and activity measurement of viral enzyme proteins

In order to develop new drugs against viral infections, it is important to understand the mechanisms of viral growth and virulence and to clarify the underlying pathogenesis. Molecular targets, such as enzymes encoded by viruses, are widely known and are often used for targeted therapy. However, in some cases, extraction of active proteins using the conventional Escherichia coli or insect cell systems can be difficult because of insolubility. To find a solution for this problem, we evaluated the possibility to synthesize active proteins by using a wheat germ cell-free protein expression system. We were able to synthesize the reverse transcriptase and HIV-1 integrase proteins in vitro.

Method

Construction of Plasmid DNA Template

We created a plasmid DNA template by cloning the reverse transcriptase gene from HIV-1 NL43-strain as well as the integrase gene from HIV-1 HXB2-strain into a pEU expression vector optimized for our wheat cell-free expression system. The p51 and p66 proteins were created to form the reverse transcriptase complex. We added a GST tag to the N-terminus of the p51 and integrase to allow for affinity purification after synthesis. For tag removal, PreScission Protease recognition sequence was added to p51 and a TEV recognition sequence was added to integrase.

Protein expression

We synthesized mRNA from the plasmid DNA template. The reverse transcriptase was coexpressed through the p51 and p66 plasmids. We created a bilayer compound for this. One of the layers had a liquid mixture of mRNA, creatine kinase, and wheat germ extract WEPRO7240G; the other layer had translation buffer SUB-AMIX SGC. After the synthesis, we performed affinity purification using the GST tag that was removed by the PreScission Protease to obtain a purified p51:p66 reverse transcriptase protein complex and integrase (figure 1). After synthesis, the integrase was solubilized through the addition of NaCl.

Reverse transcriptase activity measurement

In this enzyme reaction system, poly (A) was used as a template and oligo (dT) as a primer; in addition, DIG-labeled dUTP and biotin-labeled dUTP were incorporated into the complementary DNA (cDNA) during extension, thereby fixing the cDNA onto a streptavidin-coated plate, allowing the use of anti-DIG antibodies to detect reverse transcriptase activity.

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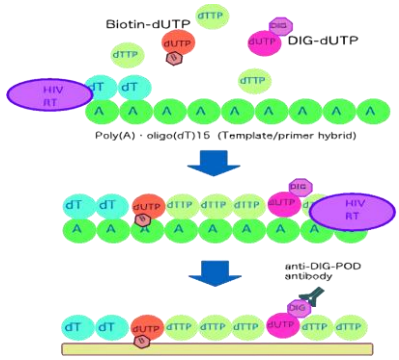

Figure 1: Diagram of the method used to measure reverse transcriptase activity.

Integrase activity measurement

Strand transfer (ST) reaction was used to detect integrase activity. The target DNA was DIG labeled and the donor DNA was biotin labeled. After the ST reaction, the DNA was fixed to a streptavidin-coated plate and integrase activity was detected by binding of the anti-DIG antibody.

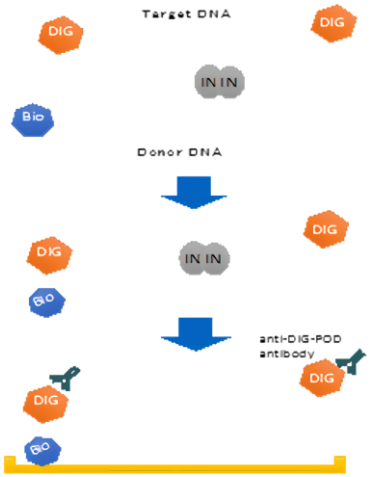

Figure 2: Diagram of the method used to measure integrase activity

Results

Reverse transcriptase p51:p66 protein complex and Integrase protein expression and purification

We were able to synthesize a soluble target protein.

We confirmed that reverse transcriptase can be prepared as a protein complex.

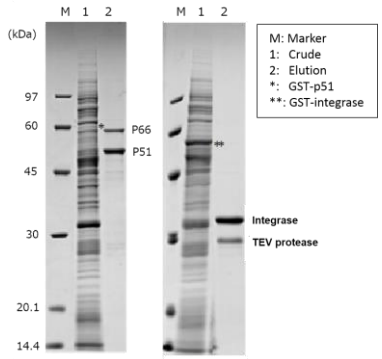

Figure 3: Reverse transcriptase p51:p66 protein complex and integrase protein expression and purification

Reverse transcriptase activity measurement

Reverse transcriptase activity was detected in the reaction solution containing mRNA. Furthermore, this activity was inhibited AZTTP addition. This confirmed that we had specifically detected the activity of the HIV-1 reverse transcriptase synthesized in vitro using the wheat germ cell-free protein expression system.

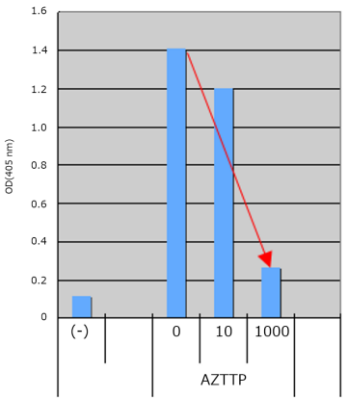

Figure 4: Activity of HIV-1 reverse transcriptase

Integrase activity measurement

Integrase activity was detected in the reaction solution containing mRNA. Furthermore, this activity was inhibited by the addition of raltegravir. This confirmed the specific detection of strand transfer activity of the HIV-1 integrase.

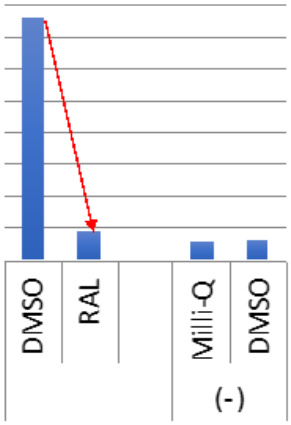

Figure 5: Integrase activity measurement results

Considerations

From the above results, we confirmed that we could easily synthesize reverse transcriptase and HIV-1 integrase proteins in vitro by using a wheat germ cell-free protein expression system. These low-expression proteins were successfully synthesized as active proteins. For reference, please also refer to the synthesis/activation examples of HIV-1 protease in the bibliography.

Acknowledgement

This paper is based on the results obtained in collaboration with Dr. Yasumasa Iwatani, Department of Infection and Immunity Research, Nagoya Medical Center, National Hospital Organization. Dr. Iwatani kindly provided the reverse HIV-1 NL43-strand-derived reverse transcriptase and HIV-1 HXB2-strand-derived integrase used for this research.

References

1. Matsunaga S, et al., A cell-free enzymatic activity assay for the evaluation of HIV-1 drug resistance to protease inhibitors. CFront. Microbiol, 2015. (6):1220

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