CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

Application Note

Synthesis of tissue plasminogen activator (tPA) protease domain with retained activity

Disulfide bonds play an important role in the formation of the extracellular domain of extracellularly secreted proteins and membrane proteins, and they are essential to the functional expression of proteins. In eukaryotes, polypeptide synthesis takes place in the reducing environment of the cytoplasm, whereas disulfide bonds are formed in the oxidative environment of the endoplasmic reticulum (ER). Protein synthesis in cell-free protein expression system is generally performed in reducing conditions, thus making it difficult for disulfide bonds to form.

Protein disulfide isomerase (PDI) and endoplasmic reticulum oxidoreductase 1a (Ero1a) are enzymes that are involved in the formation of disulfide bonds in eukaryotes. Our company introduced these proteins into a wheat germ cell-free protein expression system to test whether the synthesized proteins retained their activities.

In this experiment, we used the protease domains from tissue plasminogen activator (tPA) involved in thrombolysis (amino acid residue 297–562; six disulfide bonds; see Figure 1) as the test proteins.

Method

Protein expression

Protein expression was performed at 15°C for 20 hours with the bilayer method at a 230-µL reaction scale. The lower layer was prepared by adding PDI and Ero1a (mRNAs) to the transcription reaction mixture, which included mRNA, creatine kinase, and the wheat germ extract WEPRO7240H. The upper layer was the translation buffer SUB-AMIX-SGC (4 mM DTT or DTT free).

Activity measurement

The measurement solution used was a 50 mM Tris-HCl (pH 8.0) buffer containing 0.075% Tween 80, with a reaction substrate manufactured by Roche. To this solution, Chromozym t-PA (final concentration 0.25 mM) and 3 µL post-protein-expression were added (final volume of 100 µL). The activity was measured by calculating the absorbance (wavelength 405 nm) of the p-nitroaniline separated through digestion by the tPA protease domain and dividing the initial reaction rate (curve slope) by the amount of tPA protease domain in µg.

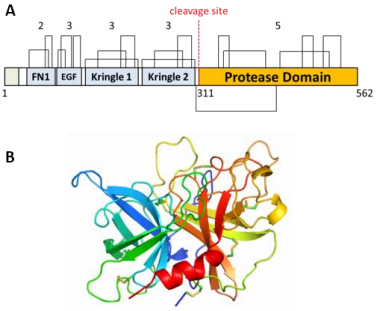

Figure 1: A: tPA domain structure and disulfide bond location B: Three-dimensional structure (PDB 1BDA) of the tPA protease domain

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Results

Protein expression

No change in solubility was observed when SUB-AMIX-SGC for normal condition (i.e., with 4 mM DTT) was used as the top layer, even after the addition of PDI and Ero1a. However, when SUB-AMIX-SGC (DTT free) was used, the solubility increased to 190%, which further increased to 380% after the addition of PDI and Ero1a (Figure 2).

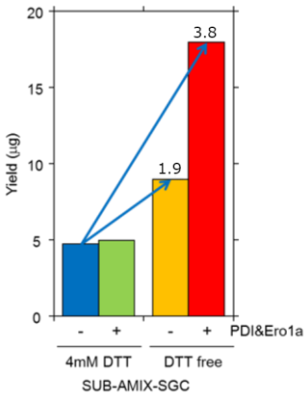

Figure 2: Solubility of tPA protease domain

Activity measurement

tPA protease domain activity measurements revealed that the results when SUB-AMIX-SGC (4mM DTT) is used as top layer, were similar to that of the negative control and no activity was observed, regardless of the presence of PDI and Ero1a.

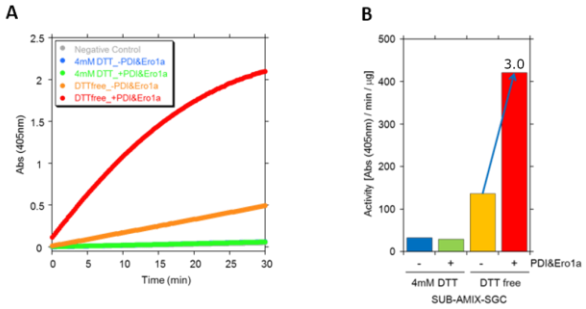

Figure 3: Activity measurement of the tPA protease domain A: time course measurement, B: Activity (Reaction rate per 1 µg)

Considerations

A possible reason for the remarkable difference between the solubility and the activity observed depending on the redox state of the upper layer and the presence or absence of added PDI and Ero1a can be explained by the fact that disulfide bonds are stable in oxidizing conditions but are quickly reduced in reducing conditions. Here we have established that it is possible to synthesize a tPA protease domain that retains its activity as it is efficiently forming disulfide bonds. We achieved this by using a bilayer method wheat germ cell-free protein expression system with a top layer of SUB-AMIX-SGC (DTT free) with the addition of PDI and Ero1a to the synthesis reactant.

References

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