CellFree Sciences

The natural power of wheat driving science

High Performance Cell-Free Wheat Germ Protein Expression System

Application Note

Synthesis of IgG Antibody Fab fragment

Abstract

Disulfide bonds play an important role in the formation of the extracellular domain of secreted as well as 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 endoplasmic reticulum (ER). Protein synthesis in cell-free protein expression systems is generally performed in reducing conditions; however, disulfide bonds can only be formed in oxidative conditions. Considering this, we are developing suitable conditions for synthesizing proteins whose activity is retained. In line with this, we introduced the enzymes involved in the formation of disulfide bonds in eukaryotes–protein disulfide isomerase (PDI) and ER oxidoreductase 1α (Ero1α)—in a wheat germ cell-free protein expression system.

In this experiment, the Fab fragment of the anti-AGIA-IgG antibody (rabbit) was used as the test protein (figure 1). Apart from the disulfide bonds in the three heavy (H) and three light (L) chains, the Fab fragment also contains a disulfide bond between the heavy and light (H-L) chains. Furthermore, several proline residues form peptide bonds in cis form. Thus, we synthesized this Fab fragment with its complicated structure and analyzed its binding ability to the AGIA antigen sequence (EEAAGIARP) using a pull-down assay.

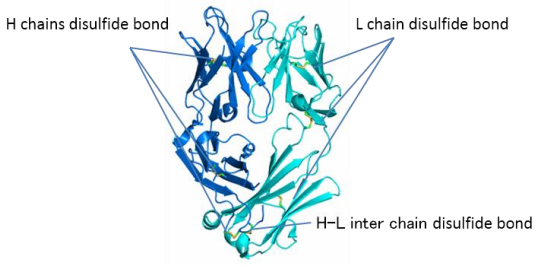

Figure1: Rabbit IgG-Rab fragment structure and disulfide bond location

Method

Protein expression

Protein expression was performed at 15°C for 20 hours with the bilayer method at a 230 µL reaction scale. Translation buffer SUB-AMIX-SGC (4 mM DTT free) was used as the upper layer. The lower layer was prepared by combining a liquid mixture of the heavy chain and light chain mRNA with creatine kinase and wheat germ extract WEPRO7240H to which PDI and Ero1α or cyclophilin B (CypB; proline residue peptide bond cis-trans isomerase were added (Figure 2).

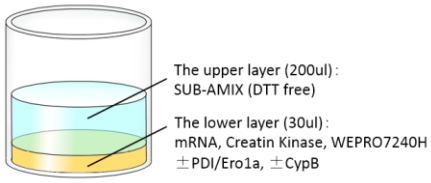

Figure 2 : Protein bilayer synthesis conditions

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Pull-down assay

The GST fusion AGIA sequence or the negative controlsequence was mixed with glutathione resin in a Tris buffer solution (50 mM Tris-HCl, pH 8, 150 mM NaCl, and 0.1% Tween80). The unrefined Fab fragment synthesis reaction solution (upper and lower layer) was added and mixed for one hour.

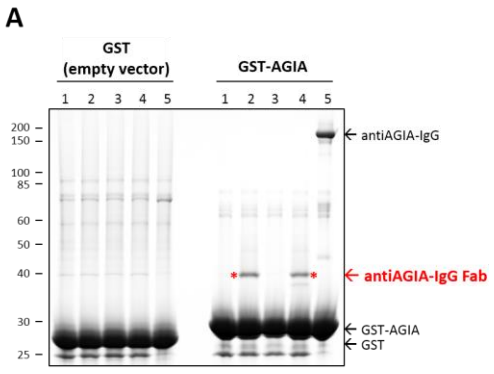
The combined Fab fragment was detected using CBB staining or Western blotting after washing the glutathione resin three times with the Tris buffer solution. Elution and SDS-PAGE were performed with SDS sample buffer.

Results

We performed the synthesis under four different conditions, with or without PDI, Ero1α, and/or CypB, Then, we checked the binding affinity of the AGIA sequence to each Fab fragment through a pull-down assay. We established that the Fab fragment (Figure3: A GST-AGIA Lane 2 and 4) synthesized by including PDI and Ero1α does bind the AGIA sequence similar to the anti-AGIA-IgG antibody (Figure3: A GST-AGIA Lane 5).

This result was confirmed using Western blotting (Figure 3: B GST-AGIA Lane 2 and 4).

On the other hand, in the absence of PDI and Ero1α, the AGIA sequence did not bind to the Fab fragment even in presence of CypB (Figure 3: A and B GST-AGIA Lane 3).



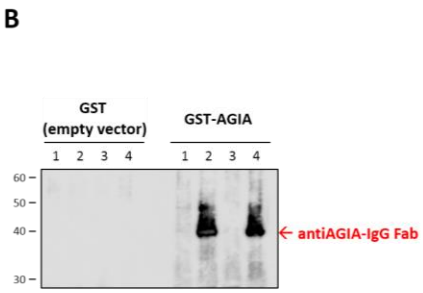


Figure 3: Pull-down assay confirming the antigen-binding ability of the fab fragment A: CBB staining results (1: Empty 2: + PDI and Ero1α, 3: + CypB, 4: + PDI, Ero1, and CypB, 5: anti-AGIA-IgG) B: Western Blotting results detected with the His-tag of the heavy chain C-terminus (Lane number as above)

Considerations

The above results demonstrate clearly that the creation of disulfide bonds by PDI and Ero1α is vital to the formation of the three-dimensional structure of a Fab fragment with antigen-binding ability. On the other hand, as CypB addition did not affect antigen-binding ability, we can deduce that, when using a wheat germ cell-free protein expression system, the cis-trans isomerization reaction of the proline residue peptide bond is not a rate-limiting step in the formation of the three-dimensional structure of the Fab fragment The underlying reason might be that the synthesis temperature of 15°C and the fact that the wheat germ cell-free protein expression system is a eukaryotic system results in a moderate rate of polypeptide chain production, giving the proline residue peptide bonds enough time to isomerize during the polypeptide chain folding reaction.

We have thus established that our method allows the synthesis of an anti-AGIA-IgG antibody Fab fragment with antigen-binding ability by adding PDI and Ero1α to a wheat germ cell-free protein expression system.

References

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