Expression of Native Human Brain Glutamte Decarboxylase 65 and Its Fragments in *Escherichia coli*

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Abstract

Autoantibodies to the glutamate decarboxylase (GAD65) are found in most insulin-dependent diabetes mellitus (IDDM) patients many years before the appearance of clinical symptoms of the disease. The structure, function, and therapeutic use of GAD65 have been precluded by insufficient quantities of purified active enzyme[1]. Previous studies have shown that the expression of GAD65 in *Escherichia coli* was mostly insoluble and of little value in immunochemical tests. In this work, ferritin heavy chain (FH) as fusion partner[2] was employed for expression of human GAD65 in *E. coli,* but overexpressed GAD65 formed mostly inclusion bodies. It seems to be caused by following reasons: 1) the MW of GAD65 (65 kDa) is too large to be folded properly in *E.coli*. 2) 15 cystein residues possessed in GAD65 lead to formation of undesired intramolecular or intermolecular disulfide bonds. Since autoantibodies to GAD65 are mostly directed to conformational epitopes, we investigated several GAD65 fragment DNA constructs designed for the production of specific GAD65 epitopes. Interestingly, the FH fusion protein of a exon15, 16 fragment of GAD65 was significantly soluble in intracellular region and detected by western blotting analysis with mAb.

References

1. Mariana L. Papouchado (1997), Expression of properly folded human glutamate decarboxylase 65 as a fusion protein in *Escherichia coli. Eur. J. Biochem.* **246**, 350-359.
2. Jeewon Lee (2002), Active human ferritin H/L-hybrid and sequence effect on folding efficiency in *Escherichia coli. BBRC.* **298**, 225-229.