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EXPRESSION AND PURIFICATION OF A MUTATED FORM OF HUMAN GAD65 FROM TRANSGENIC TOBACCO LEAVES

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Type 1 insulin-dependent diabetes (T1D) is caused by the autoimmune destruction of insulin-secreting beta cells, leading to a life-long insulin deficiency (Gepts, 1965). The young age of affected patients, the need for insulin therapy and the high prevalence of late-onset complications make T1D a major health problem.

The 65 kDa isoform of glutamic acid decarboxylase (GAD65), present on pancreatic islet beta cells, is one of the major autoantigens implicated in the development of human T1D (Baekkeskov *et al*., 1982) and it has been recently demonstrated in a Phase II Clinical Trial that two injections of the molecule can give protection to T1D. The final therapeutic aim for T1D is primary prevention because of the difficulty in identifying people at risk of T1D within the population. Vaccination studies and subsequent vaccination treatment of a large number of people would require a huge amount of purified protein, but the current production platforms are too expensive and unable to provide enough GAD65 to meet global demand.

GAD65 has previously been expressed in *Nicotiana tabacum* plants but yields were disappointing (maximum 0.25% of total soluble protein, TSP) (Porceddu *et al*., 2009; Ma S. *et al*., 2004; Wang *et al*., 2008; Avesani *et al*., 2003). In order to improve the recombinant protein expression level, we expressed a mutated form of the molecule with no catalytic activity (hGAD65mut), hypothesising that the enzymatic activity might interfere with its accumulation.

The mutated form of the molecule we used was previously described (Hampe *et al*., 2001) and it was characterised by the substitution of the amino acid residue responsible for cofactor binding in the catalytic site.

We showed that GAD65mut accumulates to higher levels in transgenic plants (2.2% TSP) than GAD65 (Avesani *et al*., 2010), suggesting that the catalytic properties of GAD65 could contribute to its poor yields.

A 1% total soluble proteins (TSP) yield of a recombinant protein in transgenic plant is considered to be the minimum required to make the extraction of a plant-derived pharmaceutical protein economically viable. Given that we obtained GAD65mut maximum yield of about 2.2% TSP, the highest-expressing GAD65mut plants allowed us to begin the set up of all steps and parameters for the purification process of the recombinant protein from plant tissue and the evaluation of plant platform capacity to meet global demand of GAD65 protein for vaccination studies and treatment, in comparison to other expression platforms.

The results obtained during the set up of the extraction and the first steps of the purification protocol are discussed.