Towards Molecular Farming of a Human Elastin-like Polymer in Plants

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Statement of Purpose: Recombinant elastin-like polypeptides (ELPs) represent one of the most fascinating example of ECM-mimicking materials, due to their unique biochemical and physical properties. In previous work, a recombinant Human Elastin-Like Polypeptide (HELP) was expressed in a prokaryotic system [1] and its enzymatically crosslinked matrices [2] studied for mechanical properties and interaction with human endothelial and aortic smooth muscle cells [unpubl. data]. This work was aimed at designing and producing HELP by recombinant DNA technology in plants. Molecular farming in plant seed is particularly attractive in view of the low investments and running costs and the possibility to obtain a pathogen-free human product which can be inexpensively stored for prolonged time [3]. HELP was selected to be the octamer of the following amino acid sequence:

Besides a cross-linking domain exploitable for the enzymatic preparation of hydrogel matrices, the monomer includes several repeats of the most structurally regular motif (VAPGVG) of human tropoelastin. The host plant was rice variety (*Oryza sativa* L., var. CR W3) unusable for food or feed purposes.

Methods:

A codon-optimized gene (HELP-R) was synthesized and cloned in a pCAMBIA1300 (www.cambia.org) derivative under the control of the rice GluB-4 glutelin promoter to give a seed-specific expression. In order to achieve the co-translational transfer of HELP into the lumen of endosplasmic reticulum, the nucleotide sequence coding for the signal peptide of GluB-4 was fused in frame with the HELP-R gene. The phosphomannose isomerase (PMI) gene driven by the CaMV35S constitutive promoter was used as marker for selection.

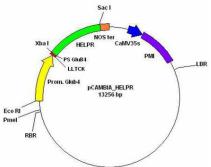


Figure 1. T-DNA composition in the expression vector used for rice transformation.

The vector was electro-porated into strain EHA105 of Agrobacterium tumefaciens; the engineered strain was

used for transformation of pre-conditioned, scutellumderived rice calluses [4]. Crude seed extracts from transgenic plants were analyzed by Western blotting using an anti human beta-elastin raised in rabbit (GenWay). Quantitative determination of HELP in a solution was achieved by DAS-ELISA, using a mouse anti-elastin monoclonal antibody (Sigma E4013) for coating and a rabbit anti-elastin polyclonal antibody for detection (Davids Biotechnologie GmbH).

Results: 153 putatively transformed plants were regenerated under selective conditions. PCR analyses showed that the T-DNA was actually present in total DNA extracted from the leaves of these plants. For 91 out of 125 tested plants an immunoreactive band of the expected mass was detected in total seed protein by Western blotting (Figure 2).

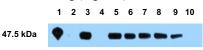


Figure 2. Lane 1: 6 μg purified HELP from *E. coli* as positive control; Lanes 2-9: 25 μg total seed protein from transformed rice plants; Lane 10: untransformed control. 1:500 anti-elastin Gen Way (0.5% milk-PBS) o/n.

Best extraction rates (>95%) were achieved with a 20mM Na₂CO₃ buffer, pH 10 for 2h. The production of HELP from seed protein extracts was eventually achieved by temperature-mediated inverse transition but so far the overall efficiency has been lower than 30%, as determined by ELISA. Further experimental data are suggesting the possibility to increase HELP production with a prior step of partial contaminant removal that could limit interactions between HELP and other proteins. At present, the use of an Ion Exchange Chromatography for contaminant retention coupled with a treatment with 25% (NH₄)₂SO₄ is being investigated.

Conclusions: With the present study, we have demonstrated HELP production in rice seed and the possibility to extract and precipitate the polymer from a starchy matrix. When the purification process will be defined, the economic feasibility of the production system will be clarified, the polymer will be characterized in detail and its crosslinked matrices will be enzymatically produced and compared to those obtained with the HELP from *E. coli*.

References:

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