Molecular Farming: expression of spider silk protein derivatives and of therapeutical recombinant antibodies in transgenic plants

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Plants and animals produce a diverse pattern of soft and hard tissues to ensure survival of the organisms under different environmental conditions. These specific properties of different tissues are based on a relatively limited set of starting materials as proteins, polysaccharides and simple minerals. Specific tissues providing mechanical features, permeability, optical properties and interaction with the environment must be developed under ambient conditions, often without external energy sources. Material scientists are increasingly willing to look to this fascinating biology for lessons in protein design and molecular evolution . Spider silk was one of the first examples in this field. The evolutionary development of the spiders was accompanied by the development of diversity, production and use of specific silks. Dragline silk, used by spiders for the frames of their webs and as safety lines, shows a combination of strength and toughness superior to high-performance synthetic fibers. Spider silk genes encode proteins composed of iterated peptide motifs. The consensus sequences are multiply repeated throughout the silk proteins. Patterns of alternating Ala-rich blocks and Gly-rich amorphous blocks provide both strength and elasticity. Such biomaterials could therefore be useful for industrial and medical purposes. Research on spider silk proteins has led to the possibility of designing genetically engineered silks according to defined material properties. Here we show the efficient and stable production of spider silk-elastin fusion proteins in transgenic tobacco and potato plants by retention in the ER. The proteins were purified by a simple method, using heat treatment and "inverse transition cycling"(Figure 1). Laboratory scale extraction of 1 kg tobacco leaf material leads to a yield of 80 mg pure recombinant spider silk-elastin protein. As a possible application, as well as to demonstrate the biocompatibility, the growth of anchorage-dependent mammalian cells on spider silk-elastin coated culture plates was compared with conventional coatings such as collagen, fibronectin and poly-D-lysine. The anchorage-dependent chondrocytes showed similar growth behaviour and a rounded phenotype on collagen and on spider silk-elastin coated plates and the proliferation was remarkably superior to untreated polystyrene plates.

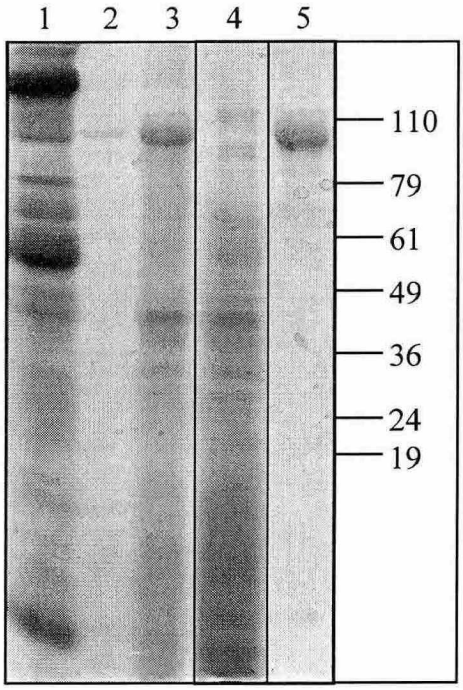

Figure 1: Purification of SO1-100xELP-proteins from transgenic tobacco plants. Coomassie stained polyacrylamide gel. 1: 15 μg of total soluble leaf protein extracted in raw extract buffer; 2: cleared supernatant of original 15 μg total soluble leaf protein after heat treatment (60 min, 95°C); 3: cleared supernatant of original 300 μg leaf protein after heat treatment; 4: cleared supernatant of original 300 μg leaf protein after heat treatment (60 min, 60°C) with 2 M NaCl; 5: solved spider silk-elastin protein pellet of original 300μg of total soluble leaf protein after heat treatment (60 min, 60°C) with 2 M sodium chloride.

Single chain Fv (scFv) as well as other recombinant antibodies became powerful tools for therapy and analysis both in human and veterinary medicine. Transgenic plants have been developed as an efficient production system of these recombinant proteins to achieve high yields at moderate prices. Especially seeds are useful organs for molecular farming because of its high protein content and the ability to keep proteins functional during extended storage at ambient conditions. Several concepts have been applied to optimise protein accumulation in seeds as the use of specific promoters and transcription enhancers. Here, we present a new strategy to remarkably enhance the expression of single chain Fv-fragemts in transgenic tobacco seeds based on protein fusion to elastin-like peptides (ELP) under control of seed-specific promoters up to 25% of TSP (Figure 2). The fusion proteins show specific activities and affinities comparable to the properties of corresponding scFv's. Thus, this strategy opens new ways to strongly improve the production of recombinant antibodies in plant seeds.

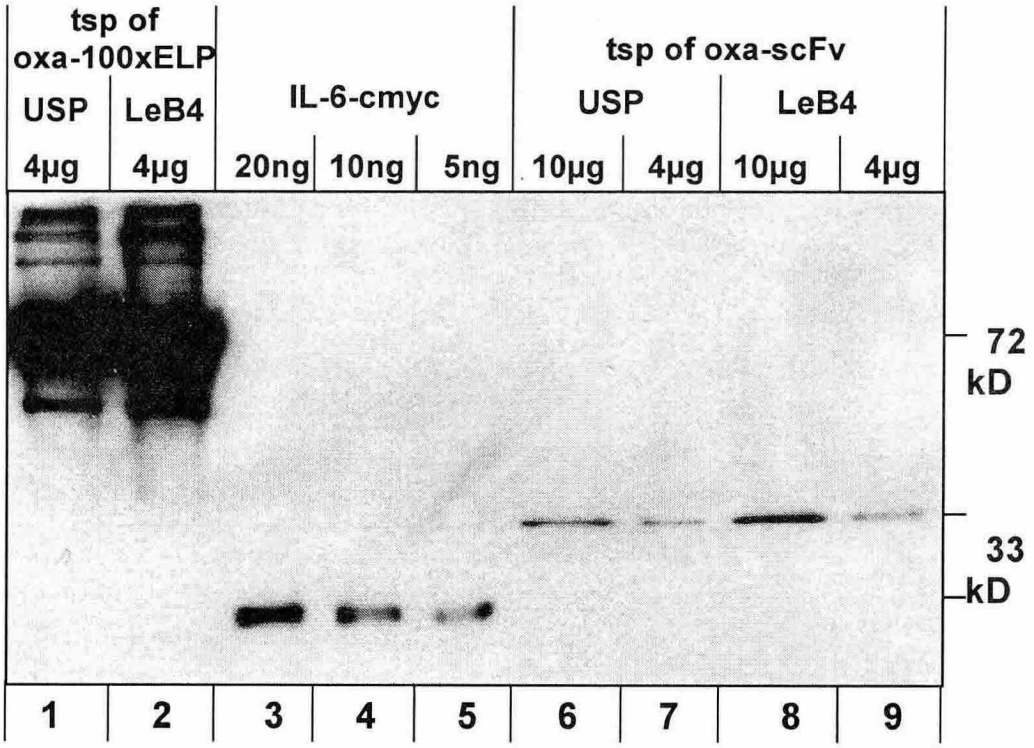

Figure 2: Expression of anti-Oxazolone scFv-ELP and anti-Oxazolone scFv in transgenic tobacco seeds under control of legumin B4 and USP promoter. Extracts, standards and markers were separated by SDS-PAGE, blotted and c-myc-tag containing proteins were detected by ECL. 1: 4μg extract Z3U1 (USP promoter-anti Oxazolone scFv-100xELP); 2: 4μg extract Z3L3 (legumin B4 promoter-anti Oxazolone scFv-100xELP); 3: 20ng IL-6-c-myc; 4: 10 ng IL-6-c-myc; 5: 5ng IL-6-c-myc; 6: 10μg extract V3U20 (USP promoter-anti Oxazolone scFv); 7: 4μg extract V3U20 (USP promoter-anti Oxazolone scFv); 8: 10μg extract V3L33 (legumine B4 promoter-anti Oxazolone scFv); 9: 4μg extract V3L33 (legumine B4 promoter-anti Oxazolone scFv).

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