

B5 Design and Expression of Novel Collagens

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Collagen is a major structural protein in the extracellular matrix of animals where it occurs mostly in the form of fibrils. The fibrils, which form by self-assembly of the triple helical collagen molecules, are of major medical importance because ectopic and excessive deposition of collagen fibrils results in fibrosis, the early stages of arterial occlusion as a prelude to heart disease and in hypertrophic scars. Collagens are also important commercially where they are used to form gelling agents in the food industry as gelatine (denatured collagen), bulking agents in cosmetics and in corrective surgery, and to assemble artificial matrices to support cell growth during tissue repair. We are presently investigating ways to modify the basic properties of collagen with a view to enhancing its ability to interact with other proteins and cells. In order to achieve sufficient quantities of modified collagen to evaluate enhanced biological properties, we are investigating various methods to express the recombinant protein.

B6 Recombinant collagen production in tobacco plants

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Because of their mechanical and biological properties and also because they are generally poorly immunogenic, collagens, notably collagen I, represent the best candidate for natural biomaterials. The principal source of collagen is bovine skin and bones. However, animal sources are now considered as risky for human health in terms of viruses and other infectious agents which can contaminate protein samples. Therefore, during the last decade, several different expression cell systems have been developed to produce safe recombinant collagen. Our objective was to use plants, which represent a cost-effective system and so far a safe host organism, as a novel expression system for the production of human collagen I. Several constructs have been engineered from the cDNA encoding human $\alpha 1(I)$ chain in order to generate transgenic tobacco plants expressing collagen I. Transformed plants were screened for the expression of collagen by Western blotting using antibodies against human collagen I. The recombinant $\alpha 1(I)$ chains were shown to fold into a stable triple helix which was disulfide-linked through its C-propeptides. N-terminal sequences of the recombinant products start with the first residue of the $\alpha 1(I)$ chain after the predicted signal peptide cleavage. Overall, the data indicate that the recombinant chains are expressed in plants as homotrimeric procollagen. Interestingly, we show that the recombinant procollagen was subsequently processed to collagen as it occurs in animals. Large amounts of triple helical collagen are now obtained by large scale purification from field grown plant material. These data suggest that plants are a valuable alternative for the recombinant production of collagen for various medical and scientific purposes.

B7 Expression of recombinant human collagens in the yeast *Pichia pastoris*

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An efficient expression system for recombinant human collagens will have numerous scientific and medical applications. However, most recombinant systems are not suitable for this purpose as such, as they do not have sufficient amounts of prolyl 4-hydroxylase (4-PH) activity. We have developed a method for producing the three major fibril-forming human collagens, types I, II and III, in the methylotrophic yeast *Pichia pastoris*. This system is based on the simultaneous coexpression of collagen polypeptide chains with the α and β subunits of 4-PH. Coexpression of the human 4-PH α and β subunits produced low amounts of an active $\alpha_2\beta_2$ enzyme tetramer. A much higher assembly level was obtained when a *Saccharomyces cerevisiae* pre-pro sequence was used to replace the signal sequence in the β subunit. The recombinant 4-PH was active in *Pichia* as the 4-hydroxyproline content of the purified recombinant collagens was found to be very similar to those of the nonrecombinant proteins. The expression levels using single-copy integrants and a 2-liter bioreactor ranged from 200 to 600 mg/l depending on the collagen type.

B8 Towards a fibrous composite with dynamically controlled stiffness: how do urchins do it?

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Sea urchins and sea cucumbers, like other echinoderms, regulate the tensile properties of their collagenous tissues by regulating stress-transfer between collagen fibrils. The collagen fibrils are spindle shaped and up to 1mm long with a constant aspect ratio of 2k. They are organized into a tissue by an elastomeric network of fibrillin microfibrils. Interactions between the fibrils are regulated by soluble macromolecules that are secreted by local, neurally controlled, effector cells. We are characterizing the non-linear viscoelastic properties of the tissue under different conditions, as well as the structures, molecules, and molecular interactions that determine its properties. In addition, we are developing reagents that will bind covalently to fibril surfaces and reversibly form crosslinks with other reagents, resulting in a chemically controlled stress-transfer capacity. The information being developed will lead to the design and construction of a synthetic analog containing fibers in an elastomeric matrix containing photo- or electro-sensitive reagents that reversibly form interfibrillar crosslinks.