Construction and bioproduction of a "green" synthetic protein-based polymer exhibiting a smart behaviour

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Natural occurring elastomeric proteins occur in a wide range of biological systems, fulfilling precise functional roles [Tatham and Shewry, 2000]. Their properties are due to the presence of short repeating oligopeptide sequences contained in fibrous proteins, such as silk fibroin (GAGAGS) and mammalian elastin (VPGVG). Elastin is widely distributed in vertebrate tissues, acting statically in dermis to resist long-term forces and dynamically in arteries to store and release energy rapidly. Natural silk from *Bombyx mori* (silkworm) has been used for centuries either in textile industry or as biomedical suture material, exhibiting impressive mechanical properties as well as high biocompatibility [Kim *et al*, 2004].

With the development of protein engineering and nano(bio)technologies in general, it is now possible to use amino acids to design and produce genetically engineered Protein-Based Polymers (PBPs) fully biodegradable that simulate the properties of natural occurring proteins . With the advance in recombinant DNA technology it is possible to precisely control the composition, sequence and length of large molecular weight PBPs [Haider *et al*, 2000]. Recombinant Elastin-Like Polymers (ELPs) are biopolymers based on the aminoacid sequence VPGXG (V-valine, P-proline, G-glycine), where X, termed the guest residue, is any naturally occurring aminoacid except proline. The most striking feature of the ELPs is their Inverse Temperature Transition (ITT) behaviour. Below a specific critical temperature (T_t) and in the presence of water they are soluble, with the polymer chains relatively extended in a disordered state and fully hydrated mainly by hydrophobic hydration. Above the T_t, the polymer chains hydrophobically fold and adopt a dynamic structure, called β-spiral, stabilized by hydrophobic contacts . The ability of ELPs to self-assemble into nanostructures in response to environmental changes allows their utilization in many devices such as microparticles for controlled drug delivery systems or nanosensors.

The polymer poly(VPAVG), a ELP where the central glycine (G) is substituted by a L-alanine (A), was chemically synthesized by Rodríguez-Cabello and co-workers and described by Urry as having thermoplastic properties. These groups reported its characterization, demonstrating its extreme biocompatibility both *in vitro* and *in vivo,* as well as the ability to self-assemble, forming microparticles that can entrap active substances during the self-assembling process [Herrero-Vanrell *et al*, 2005; Rincón *et al*, 2006].

In the present work a new thermal responsive, biologically synthesized ELP based on the $(VPAVG)_{220}$ sequence was produced, by recurring to standard molecular genetic tools and, as expected, the polymer displayed an inverse temperature transition (T_t)

which could be explored as a purification step. Additionally, the purified polymer $(VPAVG)_{220}$ showed the ability to self-associate at physiological temperature forming aggregates.
The culture media and fermentation conditions were optimized using a Central Composite Design (CCD) approach while exploring the use of low cost carbon sources like lactose and glycerol. Sequence and purity of $(VPAVG)_{220}$ was confirmed by MALDI TOF analysis and purified polymer was subjected to thermal and physical characterization. Due to its self-assembling behaviour near 34 ºC stable spherical microparticles of a ~1µm diameter were obtained, ready solubilized when a strong undercooling was achieved.
Moreover, we have constructed and produced a new set of copolymers (Silk-ElastinLike Polymers – SELPs) consisting of flexible ELP and crystalline silk-like blocks (GAGAGS) at different proportions. By this strategy it was possible to produce a variety of biomaterials with diverse physical properties, such as viscosity and gelation time depending on the number of elastin-blocks and silk-like blocks, respectively [Megeed *et al*, 2000]. The stability of these SELPs in combination with their biocompatibility and unique mechanical properties, provide the basis of their exploitation for biomedical applications.

References

Tatham, A. and Shewry, P. (2000). Elastomeric proteins: biological roles, structures and mechanisms. *TIBS*, 25, 567-571.

Kim, U., Park, J., Li, C., Jin, H., Valluzzi, R., Kaplan, D. L. (2004). Structure and Properties of Silk Hydrogels. *Biomacromolecules*, 5 (3), 786-792.

Haider, M., Leung, V., Ferrari, F., Crissman, J., Powell, J., Cappello, J., Ghandehari, H. (2000). Molecular Engineering of Silk-ElastinLike Polymers for Matrix-Mediated Gene Delivery: Biosynthesis and Characterization. *Molecular Pharmaceutics*, 2 (2), 139-150.

Herrero-Vanrell, R., Rincón, A.C., Alonso, M., Reboto, V., Molina-Martinez, I.T., Rodríguez-Cabello, J.C. (2005). Self-assembled particles of an elastin-like polymer as vehicles for controlled drug release. *Journal of Controlled Release*, 102, 113-122.

Megeed, Z., Cappello, J., Ghandehari, H. (2002). Genetically engineered silk-elastinlike protein polymers for controlled drug delivery. *Advanced Drug Delivery Reviews*, 54 (8), 1075-1091.

Rincón, A.C., Molina-Martinez, I.T., de las Heras, B., Alonso, M., Baílez, C., Rodíguez-Cabello, J.C., Herrero-Vanrell, R. (2006). Biocompatibility of elastin-like polymer poly(VPAVG) microparticles: *in vitro* and *in vivo* studies, *Journal of Biomedical Materials Research Part A*, 78A, 343-351.