COMMENTARY

Proteins from PHB granules

GEORGE GEORGIOU^{1,2,3} AND KI JUN JEONG^{1,2}

¹Department of Chemical Engineering, ²Institute for Cellular and Molecular Biology, and ³Department of Biomedical Engineering, University of Texas at Austin, Austin, Texas 78712, USA

Over the years a number of techniques that rely on the fusion of a gene to a sequence encoding a polypeptide that can be readily purified by affinity chromatography have been devised to facilitate the protein purification both at the analytical and at the industrial scale (Nilsson et al. 1997; Hearn and Acosta 2001; Jonasson et al. 2002). While these techniques had greatly expedited the production of cloned products, they are not without drawbacks. First, affinity chromatography matrices are quite costly, an issue that becomes particularly significant in large-scale applications (Hearn and Acosta 2001; Przybycien et al. 2004). Second, the fusion protein has to be proteolytically cleaved and the affinity tag must be separated from the desired polypeptide.

A number of strategies to circumvent the cost of affinity chromatography or to facilitate the proteolytic cleavage of the fusion protein have been developed. For example, Meyer and Chilkoti (1999) reported the first nonchromatographic method, termed inverse transition cycling, for recombinant protein purification by fusion with a thermally responsive polypeptide (elastin-like polypeptide [ELP]). ELP consists of repeats of the pentapeptide sequence and undergoes a reversible inverse temperature transition. When the temperature is raised above the inverse transition temperature (T_t), the normally soluble ELP fused polypeptides form insoluble aggregates which can be separated by simple centrifugation. Subsequently, the aggregate can be resolubilized easily by a temperature shift below T_t . Recently, Chen's group (Stiborova et al. 2003) introduced affinity-modified ELPs, including imidazole-grafted ELPs for the precipitation of His-tagged proteins, thus providing an effective alternative to immobilized metal affinity chromatography. Along similar lines,

Mattiasson's group (Kumar et al. 2003) reported new affinity precipitation reagents based on the thermo-responsive polymer poly(N-isopropylacrylamide) modified with vinylimidazole metal ion chelating groups to generate thermoresponsive metal affinity precipitants. At the other end, the intein technology enables the self-cleavage of protein fusions. Inteins are the protein analogs of self-splicing RNA introns, as they post-translationally excise themselves from various precursor proteins. The ability of inteins to catalyze a precise peptide bond cleavage in a controllable manner can eliminate the use of protease for the removal of a fusion tag during the recovery steps and also a subsequent protease removal step which potentially reduces the cost of a large-scale purification process for a recombinant protein product.

In this issue of *Protein Science*, Banki et al. (2005) have now pushed the frontiers of gene fusion technology several steps forward. In this technology, precipitation of intein fusion proteins is accomplished by affinity binding to insoluble particles produced by the cell itself. Following precipitation, self-cleavage of the intein results in the release of the purified polypeptide. Banki et al. (2005) capitalized on phasins, proteins that bind tightly to polyhydroxybutyrate (PHB) granules that can be readily separated from lysed bacteria. In their strategy, cells are first engineered to produce PHB granules to which the phasin-intein fused target protein binds. Following cell lysis, the PHB granules are separated by centrifugation, and the soluble target protein is recovered following the intein-mediated self-cleaving reaction that is triggered by adjusting the pH. Banki et al. (2005) reported the purification of several recombinant proteins including maltose binding protein, β -galactosidase, chloramphenicol acetyltransferase, and NusA, with yields ranging from 30 to 40 mg/L culture.

Polyhydroxyalkanoates, including PHB, are storage polymers produced by various bacteria when the culture environment is not optimal for growth. During the past decade, polyhydroxyalkanoates have attracted

Reprint requests to: George Georgiou, Department of Chemical Engineering, University of Texas at Austin, Austin, TX 78712, USA; e-mail: gg@che.utexas.edu; fax: (512) 471-7963.

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commercial and academic interest as a source for renewable and biodegradable plastics (Lee 1996; Steinbuchel and Hein 2001). In addition, PHB granules have been used as support materials for protein chromatography and immobilization. For example, Moldes et al. (2004) used PHB granules for the immobilization of recombinant proteins via the fusion with a novel tag (BioF). The BioF consisted of an N-terminal functional domain of phasin enabling the target fusion protein to attach to PHB granules within the host cell. After separation of the PHB granules by mild centrifugation (4000g for 30 min), the BioF tag-fusion protein could be recovered by detergent treatment such as Triton X-100 or sarkosyl. However, many soluble proteins cannot withstand detergent solubilization. Banki et al. (2005) cleverly circumvented this problem by employing intein-mediated self-cleavage to recover the target protein. Importantly, intein cleavage occurs at mild conditions, reducing the risk of denaturation or covalent modification of the protein via processes such as deamidation, etc.

What's next? First, it has to be shown that cells that produce PHB and intein fusions can be grown to high cell densities, as required for many preparative applications, especially for bioprocessing purposes. Technologies for the efficient production of PHB by engineered bacteria have been perfected, and it is now possible to produce up to 160 g of PHB per liter of culture grown to a cell density of 200 g DCW per liter (Lee and Choi 2001). However, will the combination of PHB production and phasin-intein synthesis cause excessive cellular stress and result in reduced yields of proteins at the industrial scale? Second, can this method be employed for the production of proteins with disulfide bonds? Many proteins of biotechnological interest are normally secreted and contain disulfide bonds, which do not normally form in the reducing environment of the cytoplasm. Mutational inactivation of the pathways responsible for protein reduction in the cytoplasm, namely the *trxB* and *gor* genes encoding thioredoxin reductase and glutathione reductase, respectively, allows the formation of disulfide bonds in the cytoplasm thus enabling the oxidative folding of cytoplasmically expressed complex proteins such as tissue plasminogen activator (Bessette et al. 1999). Nonetheless, the ability to produce PHB granules in strains with oxidizing cytoplasm will have to be shown. Third, there are over 140 possible constituent monomers of polyhydroxyalkanoates, and consequently, granules of different composition, size, and morphology can be produced (Aldor and Keasling 2003). Since protein

yield is affected by the PHB granule composition and morphology, it would be interesting to engineer cells that produce the optimal polymer for protein expression applications.

It is noteworthy that all the steps required for the preparation of the soluble polypeptide product by the phasing-intein fusion technology are compatible with robotic implementation. Consequently, the technique developed by Wood and coworkers (Banki et al. 2005) appear to be well suited for high throughput proteomics applications. In addition, it is well suited for bioprocessing purposes, where it can provide a distinct cost advantage by circumventing the need for a chromatography separation. We, therefore, expect that the work of Banki et al. (2005) will soon assume a prominent position in the protein expression and engineering toolbox.

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