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Commentary

Genetic Engineering of Protein-Based Polymers: Potential in Controlled Drug Delivery

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The majority of the advanced drug delivery systems contain polymeric biomaterials as their central component. Whether commercially available or in the research stage, by and large biopolymers for drug delivery are synthesized using traditional synthetic methods which result in the production of polymers with heterogeneous molecular weights, and in the production of monomer sequences and compositions which can only be defined in terms of statistical distribution. Heterogeneity in molecular weight, composition, sequence, and stereochemistry influences the function of the biomaterial in drug delivery. For example water soluble polymeric drug carriers must have a narrow optimal molecular weight range, below which polymer molecules are eliminated too rapidly from the blood stream, and above which they are chronically retained in some organs such as liver, spleen, and bone marrow (1). The location and sequence of the recognition sites within the macromolecule affect the biorecognition of the polymer by the biological enzymes or receptors, and consequently influence drug targeting and release (2). Stereochemistry of copolymers of lactic-glycolic acid influences the rate of degradation and drug release from these systems (3). Therefore a higher degree of control over the molecular weight, composition, sequence, and stereochemistry of the bioploymer may have a profound effect on the biological fate and the function of the biomaterial as the drug carrier.

The purpose of this commentary is to introduce pharmaceutical scientists to an emerging technology that promises to provide unique potential to the field of drug delivery. A list of references is provided with more detailed information for critical review. This new technology renders a higher degree of control over the macromolecular structure of synthetically designed proteins using genetic engineering techniques. Methods for the successful design and biological synthesis of high molecular weight structural protein polymers were first developed in 1986 (4), and have been shown to be generally applicable to the synthesis of a variety of proteins consisting of repeating blocks of amino acid sequences (5–7). Precise control over the biomaterial structure through sequence specification is the key advantage of the new synthetic methodology. As an example, a biologically synthesized polymer comprising 36 repeats of the octapeptide sequence—$(AlaGly)_3GluGly$—was designed to adopt folded-chain lamellar architecture in the solid state, with the oligo(alanylglycine) elements forming β-strands and the periodic glutamic acid residues positioned in reverse turns at the lamellar surface (8). Spectroscopic and X-ray diffraction analyses of the polymer were found to be fully consistent with the expected structure, confirming the application of biological synthesis to control the solid-state structure of macromolecular materials.

Control over the three dimensional structure of the polymeric carrier has implications in the successful design of novel drug delivery systems. For example it has been shown that the α-helix content of a series of chemically synthesized cationic copolymers of L-lysine and L-serine (PLS) increases their affinity for a hammerhead ribozyme (R32), an antiviral antisense molecule (9). The higher percentage of the α-helical content of the copolymer in the PLS-R32 complex, resulted in a better stability of the nucleic acid in human serum. Genetic engineering offers a higher degree of control over the three dimensional structure of the polymeric carriers. Therefore this technology has a potential in the design of drug delivery systems where the affinity of drug molecules (such as nucleic acids) with the polymer needs to be controlled.

By changing the composition of amino acid sequences in protein polymers, it is possible to control drug loading and release from such biomaterials. Silk-elastinlike protein (SELP) copolymers (Protein Polymer Technologies, Inc., San Diego, CA) are a family of genetically engineered protein polymers consisting of silklike and elastinlike blocks in various block lengths and compositional ratios (10–12). The nature of the elastinlike blocks, and their length and position within the monomers influences the water solubility of the SELP polymers. Decreasing the length of the silklike block domains, while maintaining the length of the elastinlike block domains further increases the water solubility of the polymers. Some SELP's undergo an irreversible solution to gel transition under physiological conditions, and the rate of gelation may be influenced

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by the SELP composition. This phenomenon has been exploited for the potential of this family of biomaterials for the controlled release of bioactive compounds (13).

Molecular structure dictates the physicochemical properties of the macromolecules. For example it has been shown in a chemically produced sequential polypeptide (14), that simple changes in the amino acid residues of the basic protein-based polymer $(Val^1Pro^2Gly^3Val^4Gly^5)_n$ result in dramatic changes in the value of T_t, i.e., the temperature at which the inverse temperature transition begins as the temperature is raised (The process where an increase in temperature results in the hydrophobic folding and assembly of amino acids within the protein structure is referred to as an inverse temperature transition (15)). Substitution of the valyl residue with the side chain $-CH(CH_3)_2$ at position four by an isoleucyl residue with the side chain $-CH(CH_3)CH_2CH_3$ resulted in a decrease in the value of T_t by 15°C, while substitution with an alanyl residue with the side chain, $-CH_3$ causes the value of T_t to be raised by more than 20°C (14), indicating that the change in the temperature of the inverse temperature transition is in proportion to a change in hydrophobicity. Even more precise engineering of the protein-based polymers through biological synthesis provides a means by which systematic modifications can be made to the biomaterial to affect its physicochemical properties under different environmental stimuli such as changes in pH, pressure, temperature, incident light, and reduction potential.

In addition to the amino acid composition, the amino acid sequence is an important parameter that influences the properties of protein polymers. It has been demonstrated that control over the resorption rate of a series of genetically engineered silk elastinlike copolymers was achieved by precisely controlling the number or the length of silklike and elastinlike blocks contained per repeating domain, and not simply by controlling the compositional ratio of these blocks in the final copolymer (16). Such control over the bioresorption of the biomaterial can be exploited for the design of novel biodegradable drug delivery systems, where small changes in the degradation rate are required to achieve the desired drug release pattern.

Introduction of recognition sites is another method by which the functional utility of macromolecules can be improved. Conjugation of recognition sites to otherwise inert macromolecules has proved to be an effective strategy for site-specific (17), and targeted (18) drug delivery. The synthetic methodology used to introduce the recognition sites however, often presents formidable challenges to the successful development of such biopolymers. As an alternative, recombinant DNA technology provides a means by which recognition sites can be incorporated into the biopolymer in a precisely controlled fashion. One example is ProNectin™ F (Protein Polymer Technologies, Inc., San Diego, CA), the first commercially available genetically engineered protein polymer, used as a coating reagent for cell cultureware. It is designed using two oligopeptide blocks, i.e., a six amino acid block providing the structural properties of silk, and a seventeen amino acid block containing the Arg-Gly-Asp sequence of human fibronectin providing the cell attachment activity of fibronectin. The blocks are precisely configured into a repeating gene monomer such that one cell-attachment block occurs after every nine silklike blocks:

$[(GlyAlaGlyAlaGlySer)_9GlyAlaAlaValThrGlyArgGlyAspSer-ProAlaSerAlaAlaGlyTyr]_n$

This monomer is repeated 13 times to yield a protein polymer chain of 980 amino acids in length with a molecular weight of 76,000 daltons. In the design of ProNectin™ F, the structural properties of the hexamer silk block (e.g., structural stability, thermal and chemical resistance, the ability to adsorb to hydrophobic surfaces), are combined with the biorecognition properties of the cell attachment blocks to provide a biorecognizable polymer film suitable for cell cultureware. ProNectin™ F demonstrates both the feasibility, and the functional utility of introducing recognition sites to polymeric carriers using recombinant DNA technology. This concept has important implications in targeted drug delivery where various informational amino acid sequences, such as those present in the extracellular matrix, can be introduced into the polymeric carrier in a controlled fashion. In addition, protein polymers containing informational amino acid sequences can be genetically tailor-made to produce new biologically active polymers with a higher activity than their synthetic counterparts. New designs can be based on results generated from previous studies for example, showing that poly (Arg-Gly-Asp) augments the inhibition of tumor lung metastases, when compared to their random poly (Arg, Gly, Asp) counterpart, or a monovalent unit of Arg-Gly-Asp alone (19). Another situation where the introduction of biorecognition sites to the protein polymer can be useful is in the areas of cell implants for drug delivery and tissue engineering. The normal growth of implanted cells is only possible when they are viable. Cell viability in part depends on its ability to grow and spread. Protein polymers containing informational amino acid sequences can be engineered to provide for cell spreading and growth, with a desirable oxygen and nutrient intake and drug release profile.

The successful biological synthesis of novel protein-based polymers requires knowledge about the present limitations of this technology. Although incorporation of some non-natural amino acids has been successful (20), the biological expression is predominantly limited to the 20 natural amino acids in the L-configuration. Other important factors that must be considered in the synthetic scheme are the consideration of the host cell preference for codons (not every synthetic gene will necessarily lead to the desired amino acid sequence), and potential untoward effects on expression such as intramolecular folding of messenger RNA resulting in impaired translation (6). Important issues regarding these limitations, the state-of-the-art materials produced by this technology, cost, and the ability to manufacture these products have been reviewed elsewhere (5,10).

In summary, the precise control over the molecular weight, composition, sequence, and stereochemistry of biopolymers using genetic engineering techniques, provides an enormous potential for the design of new materials for use in drug delivery systems. The successful exploitation of this concept however, relies on valuable lessons learned from drug delivery research up-to-date, and recognition of the current limitations of our knowledge about the novel technology.

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