

**POLYMERIZABLE COMPOUNDS AND
METHODS FOR PREPARING SYNTHETIC
POLYMERS THAT INTEGRALLY CONTAIN
POLYPEPTIDES**

DESCRIPTION

1. Technical Field

The present invention relates to compounds and methods used for the de novo synthesis of organic polymers that contain polypeptides as an integral part of their backbone structure.

2. Background Art

A reaction fundamental to polymer chemistry is the initiation of end-to-end covalent linkages between soluble organic monomeric compounds (monomers) leading to the formation of larger polymeric molecular structures (polymers). Synthetic polymers may be formed from a single monomeric species (homopolymers) or from a mixture of different monomers (copolymers). Linear, branched, or cross-linked structures are possible. By varying the chemical composition or ratios of the monomers, it is possible to form either soluble or insoluble polymers which comprise a broad range of chemical and physical structures. For example, water-soluble monomers (such as acrylamide) may be homopolymerized to form water-soluble homopolymers. They may also be copolymerized with less water-soluble monomers (such as N-alkyl or N,N-dialkyl acrylamides) or with cross-linking monomers (such as N,N'-methylenebisacrylamide) to form water-insoluble copolymer structures. Some water-soluble monomers (such as hydroxyethyl methacrylate or acrylonitrile) may be homopolymerized to form water-insoluble homopolymers.

In the fields of biochemistry and immunology, water-insoluble polymers (such as polyvinyls, polyacrylamides, or polydextrans) have been commonly used as solid-phase supports with passively adsorbed, physically entrapped, or covalently linked proteins in affinity chromatography, enzyme immobilization, and immunoassays. To date, the documented covalent coupling of a polypeptide to a polymer has occurred under circumstances in which the polypeptide was provided in soluble form and the polymer was provided as a preformed soluble or insoluble polymer material.

For purposes of affinity chromatography (Affinity Chromatography and Related Techniques, Proceedings of the Fourth International Symposium, Veldhoven, The Netherlands, June 22-26, 1981, eds. T. C. J. Gribnau, J. Visser, and R. C. F. Nivard, Elsevier Scientific Publishing Co., N.Y., 1982), antibodies can be covalently bonded to cyanogen bromide-activated beads of Sepharose® 4B (Pharmacia Fine Chemicals AB, Uppsala, Sweden) or beads of cross-linked acrylic polymers (U.S. Pat. No. 3,957,741). The immobilized antibodies can then be used to specifically bind antigens to the solid surface followed by extensive washing to remove other adsorbed substances. Subsequently, the bound antigens can be eluted from the antibody/polymer matrix by treatment with chaotropic agents, high salt, or low pH buffers. Antibodies have also been confined within capsule membranes for use in affinity chromatography (U.S. Pat. No. 4,257,884).

In certain chemical processes, immobilizing enzymes on insoluble matrices provides a convenient method of selectively controlling a chemical reaction. For example, enzymes entrapped within, or bound to the surface

of, polymer beads can be added to reactants in solution for a discrete period of time and then selectively removed by physical procedures, such as centrifugation. Alternatively, chemical reactants in solution may be brought into controlled physical contact with enzymes by chromatography through columns comprised of polymer beads to which enzymes have been covalently coupled. For example, see U.S. Pat. No. 4,195,129. Graft copolymerization has also been employed as a means of enzyme immobilization. For example, D'Anguio et al. (Biotechnol. Bioeng., vol. 22:2251, 1980) describe graft copolymerizing vinylated enzymes to a preformed polymeric surface.

In immunoassays (see Campbell, D. H. and Weliky, N., Methods in Immunology and Immunochemistry, Editors: Williams and Chase, Vol. 1, Academic Press, N.Y., 1967), antibodies or antigens have been passively adsorbed to plastic surfaces, e.g., the wells of microtiter plates or plastic beads (U.S. Pat. No. 4,225,784) or to latex particles. The solid-phase antibody/polymer matrix provides a selective binding surface which, following an appropriate reaction, can be washed to separate bound from unbound reactants. Alternative uses include (a) the covalent binding of antigens or antibodies to latex beads (U.S. Pat. No. 4,181,636) or high refractive index particles (U.S. Pat. No. 4,401,765) to measure agglutination reactions, or (b) the binding of antibodies to fluorescent polymer beads to provide specific tags for cell surface antigens (U.S. Pat. No. 4,166,105).

While these insoluble polymers are of utility in providing a surface upon which selective biochemical or immunological reactions can occur, the polymers are of limited value in that the spacing, steric accessibility, and number of protein molecules bound per unit length of polymer cannot be precisely or reproducibly controlled. Lot-to-lot variation is commonly encountered during the manufacture of such solid-phase polypeptide/polymer matrices. In certain end-use applications where reproducibility and standardization are essential (e.g., immunoassays), this variation in composition of the solid-phase polymer/polypeptide matrices presents a critical problem. Consequently, there is a need in the art for a method to specifically tailor or molecularly engineer polymer compounds incorporating controlled quantities of polypeptides.

DISCLOSURE OF THE INVENTION

Briefly stated, the invention discloses a method for preparing synthetic polymers which integrally contain specific polypeptides as part of their structure. Essential features of this method include (a) the covalent linkage of soluble organic monomers to selected polypeptides to form soluble monomer/polypeptide conjugates, followed by (b) the copolymerization of these conjugates with nonderivatized monomers to form synthetic copolymers that integrally contain polypeptides in their structure. Utilizing controlled chemical synthesis, comparable to that conventionally practiced in the polymer chemistry field, it is possible to control the spacing, steric accessibility, and number of polypeptide molecules along the backbone of the polymer, providing unique advantages for certain end-use applications.

Accordingly, the present invention is directed to (1) monomer/polypeptide conjugates used in making synthetic polymers that integrally contain one or more polypeptides, (2) methods of making the monomer/polypeptide conjugates, (3) methods of making poly-