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Commentary

Soluble Self-Assembled Block Copolymers for Drug Delivery

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INTRODUCTION

Soluble block copolymers may self-assemble into novel supramolecular structures, which possess functional properties for drug delivery (1–5). These unique molecular architectures are being researched for the delivery of anticancer drugs, proteins and plasmid DNA (1–5). A major consideration of these drug delivery systems is their nanoscopic dimensions, which may yield advantages in terms of drug targeting, safety and development. Moreover, there has been substantial progress in their chemistry, and we can now envision biocompatible, biodegradable synthetic analogues of biological transports systems, lipoproteins or viruses.

Several model systems have broad impact and appeal, and these will be discussed. Detailed reviews of self-assembled block copolymers (micelles) are available in the literature (1–5).

A Micelle-Forming Block Copolymer-Drug Conjugate

A poly(ethylene oxide)-*block*-poly(aspartic acid) (PEO-*b*-PAA)-doxorubicin (DOX) conjugate self-assembles into a spherical micelle-like structure in water (6,7). It has a nonpolar core composed of a PAA block with attached DOX, and a hydrophilic shell composed of PEO. Its diameter is ca., 30 nm, based on dynamic light scattering measurement, corresponding to the size of lipoproteins and viruses (6). The attachment of DOX onto PEO-*b*-PAA occurs at high levels without a loss of water solubility. A loss of water solubility often limits the degree of drug substitution of soluble synthetic polymers. The micelle-like structure of a PEO-*b*-PAA-DOX conjugate is held tightly together by self-association of DOX in the core region, evidenced by quenching of drug fluorescence (7). Even in blood, a micelle-like structure of a PEO-*b*-PAA-DOX conjugate breaks apart gradually (hours). This unique stability toward break-up enables PEO-*b*-PAA-DOX conjugate micelles to have a prolonged blood half-life, as opposed to rapid renal clearance of its unimer (8). PEO plays a recognized role in blood, preventing protein adsorption and cellular adhesion, steps that precede phagocytosis by cells of the mononuclear phagocyte system. An inordinately dense layer of PEO (polymer brush) masks the nonpolar core of drug. PEO-*b*-PAA-DOX conjugate micelles, therefore, passively accumulate at solid tumors by the enhanced permeability and retention effect (9). Conjugated DOX on PAA plays no direct role in antitumor activity expressed in murine tumor models (10). It was found instead that unconjugated DOX is held tightly in cores of PEO-*b*-PAA-DOX conjugate micelles and carried to tumors where it exerts antitumor effects.

Synthetic micelle-like structures of block copolymers may pose as functional analogues of biological transport systems, such as low-density lipoprotein. A major function of plasma lipoproteins is the solubilization of lipids, and synthetic micelle-like structures of block copolymers may play an analogous role for water-insoluble drugs. Synthetic micelle-like structures of block copolymers may deliver unconjugated drug to solid tumors without an excessive loss of drug. Lastly, synthetic micelle-like structures of block copolymers may be more easily scaled up and less costly than lipoproteins for drug delivery.

A Family of Micelle-Like Structures of PEO-Block-Poly(L Amino Acid) (PLAA)

A family of micelle-like structures from PEO-*b*-PLAA is being investigated for drug delivery (1,2). The side-chains of a core-forming PLAA may possess drugs, e.g., DOX and cisplatin. For the solubilization of drugs without covalent bonds, side chains may possess nonpolar elements, e.g., benzyl alcohol, an aromatic moiety (11). PEO-*block*-poly(β benzyl L aspartate) effectively solubilizes aromatic drugs, such as indomethacin (12). The side chains may also possess nonpolar elements from serum lipoprotein, e.g., fatty acid, for aliphatic drugs (13).

With an array of PEO-*b*-PLAA micelles, structure-property relationships may be established. This may provide insight on major properties of PEO-*b*-PLAA micelles, drug solubilization, stability toward break up and drug release. This may also provide insight on the biocompatibility of PEO-*b*-PLAA. The biodegradability of self-assembling PEO-*b*-PLAA needs to be examined. The immunogenicity of PEO-*b*-PLAA must be researched initially in murine models, especially for PLAA blocks with more than two kinds of L amino acids (14). Block copolymers may act as an immunological adjuvant (15). Major principles of interaction with living systems that are satisfied by soluble polymers and polymer-drug conjugates may be extended to an array of self-assembling PEO-*b*-PLAA and further elaborated (16).

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Micelle-Like Structures of Block Copolymers Solubilize Taxol, an Important Anti-Cancer Drug

PEO-*block*-poly(D,L lactic acid) (PEO-*b*-PDLLA) self-assembles into a micelle-like structure in water that effectively solubilize taxol, a promising anticancer drug for breast and ovarian cancers (17–19). The water solubility of taxol is poor (1 μM), but PEO-*b*-PDLLA micelles solubilize taxol at 20 mg/ml in water without precipitation upon dilution (17). A ^{1}H NMR spectrum of PEO-*b*-PDLLA and taxol in D_2O indicates that taxol is located in the PDLLA core. PEO-*b*-PDLLA micelles rapidly release taxol in the presence of plasma, and taxol binds serum proteins, such as high-density lipoprotein (18). Hence, the biodistribution of taxol is similar to that of its usual parenteral formulation in a murine model (19). However, taxol-loaded PEO-*b*-PDLLA micelles express less toxicity and improved antitumor activity.

A mixture of Cremephor EL® (polyethoxylated castor oil) and ethanol solubilize taxol in the currently used parenteral formulation. There is considerable interest in alternatives, owing to serious side effects associated with the administration of a mixture of Cremephor EL® and ethanol, such as hypersensitivity, and its widespread use for water-insoluble parenteral drugs. PEO-*b*-PDLLA may be a safer option. PEO is a nontoxic polymer. PDLLA is a biodegradable polymer, which has proven to be nontoxic. Both polymers are approved parenteral use.

Antisense Oligonucleotide and Plasmid DNA May Participate in Self-Assembly of Soluble Block Copolymers

Instead of self-assembly due to hydrophobic interaction of amphiphilic block copolymers, self-assembly of PEO-*block*-poly(L lysine) (PEO-*b*-PLL) and oppositely-charged antisense oligonucleotide or plasmid DNA can take place with cooperative electrostatic interaction as a driving force, resulting in higher-ordered structures with nanoscopic dimensions and a spherical shape (5,20–23). The self-assembly of PEO-*b*-PLL with antisense oligonucleotide or plasmid DNA takes place without a loss of water solubility at an equimolar ratio of lysine to phosphate groups. At this ratio, PLL combines with antisense oligonucleotide or plasmid DNA with a loss of water solubility. It was proposed that PEO-*b*-PLL assembles with anti-sense oligonucleotide or plasmid DNA into a core-shell structure, where complexed PLL and "guest" reside in a core (22,23). Accordingly, PEO-*b*-PLL protects plasmid DNA from both thermal disruption of base stacking and degradation by nuclease.

Owing to nanoscopic dimensions and protection of plasmid DNA afforded by PEO-*b*-PLL, a core-shell complex may act as an "artificial virus," which may influence the biodistribution of plasmid DNA. It is noteworthy that the delivery of plasmid DNA to solid tumors may be favored by a reduction in the dimensions of a gene delivery system (24). Attachment of pilot molecules, e.g., a monoclonal antibody, onto chain ends of PEO may also improve delivery.

Self-Assembled Block Copolymers May have Surface Elements for Site-Specific Drug Delivery

Since "guest" molecules reside in a core region of self-assembled block copolymers, they are shielded from an aqueous milieu of a biological host by a layer of PEO. Thus, functional properties of a core are clearly separated from the functional properties of a layer of PEO, and each region can be tailored independently. It is well established that PEO repels proteins and cells. Attachment of PEO on drug delivery systems yields prolonged blood circulation times and may yield enhanced accumulation at solid tumors (25). Can a layer of PEO on self-assembled block copolymers acquire additional functional properties for drug delivery?

With newly synthesized heterobifunctional PEO, pilot molecules may be tethered at surfaces of self-assembled block copolymers for recognition and more selective drug delivery (26–28). Pilot molecules such as insulin or an antibody mediate the delivery of micelles composed of PEO-*b*-poly(propylene oxide)-*b*-PEO (Pluronics®)(26,27).

Pluronics® micelles conjugated with insulin can enhance the accumulation of fluorescein isothiocyanate in organs of mice. A brain-specific antibody (against α_2-glycoprotein) is able to selectively mediate the delivery of haloperidol to brain tissue, resulting in an enhancement of drug effect (26,27).

Block copolymer micelles have reactive aldehyde on their surfaces, which react with lysine residues of proteins (26–28). Sugars play a role in receptor-mediated cell binding, and a sugar has recently been attached on a chain end of a PEO, with the other chain end available for attachment of a hydrophobic or charged block. Similarly, biotin has been attached on a chain end of PEO, and biotin may strongly bind monoclonal antibody-streptavidin conjugates.

Alternatively, poly(N-isopropylacrylamide) (PNIPAM) may take the place of PEO in a self-assembling block copolymer (29–31). PNIPAM is hydrated at 25°C, and it repels proteins and cells. But above 32°C, its lower critical solution temperature (LCST), PNIPAM dehydrates and becomes hydrophobic, and it interacts with proteins and cells. Its LCST can be adjusted close to 37°C by the random copolymerization of NIPAM with other monomers. PNIPAM micelle-like structures may circulate in blood until they passively reach target sites, e.g., solid tumors. Their cellular uptake may be induced after local elevation of temperature. This novel approach may be considered a "double" form of site-specific drug delivery.

Pluronics® Enhance p-Glycoprotein-Mediated Transport of Drugs

Substantial evidence points toward the inhibition of P-glycoprotein by Pluronics® (32–34). Certain Pluronics®, e.g., P85, strikingly increase the cytotoxicity of drugs, e.g., daunorubicin, against multidrug cells overexpressing P-glycoprotein (32). It appears that unimers of Pluronics® are able to inhibit P-glycoprotein. The mechanism of inhibition is unclear, but it may be related changes at a membrane level induced by Pluronics®. This may inhibit P-glycoprotein or enhance cellular uptake of drugs. In a cell model of the blood-brain barrier, Pluronics® (P85) inhibit P-glycoprotein as a unimer, increasing absorption of rhodamine 123 (33). Similarly, Pluronics® (P85) inhibit P-glycoprotein in Caco-2 monolayers (33). Pluronics® are better than other nonionic detergents noted for their inhibition of P-glycoprotein.

CONCLUSIONS

Since self-assembled block copolymer-drug conjugates were first proposed as synthetic analogues of lipoproteins, there

have been promising advances in this area of drug delivery (35). By mimicking nanoscopic supramolecular core-shell structures of lipoproteins and viruses, soluble self-assembled block copolymers may possess multiple functional properties, e.g., high carrying capacity of guests, prolonged blood circulation half-life, overcoming of membrane barriers and cell recognition. The micelle-like structures of amphiphilic block copolymers may eventually play a role in the solubilization of water-insoluble drugs beyond that of the established Pluronics®. The growing family of self-assembling PEO-*b*-PLAA and also PEO-*b*-PDLLA are noteworthy in this regard.

Supramolecular core-shell structures of PEO-*b*-PLL with antisense oligonucleotide, proteins or plasmid DNA may pose as synthetic analogues of viruses, with potential for site-specific delivery. There is clearly a vital need of targetable gene delivery systems beyond viral vectors.

Amphiphilic block copolymers may also form nanospheres, which may play a role in drug delivery [36]. The nanospheres are larger than the micelle-like structures of block copolymers (>100 nm), but they are also spherical. X-ray photon-electron spectroscopy reveals a surface layer rich in PEO on nanospheres of amphiphilic block copolymers. Their larger dimensions may be a detriment for drug targeting, but they possess interest for controlled drug release.

Thus, self-assembly of block copolymers may provide micelle-like structures or nanospheres. What determines which supramolecular structure is formed? Clearly, the structure of a block copolymer is important. The presence of drug may also have an effect, but this has not been studied in detail. The self-assembly conditions of block copolymers must also be considered.

Finally, an expanded interest in self-assembled block copolymers may lead to a useful class of nanoscopic drug delivery systems with unparalleled functionality, safety and effectiveness.

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