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Varying Polymer Architecture to Deliver Drugs

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Abstract

Variable architecture polymers are of considerable interest for the delivery of therapeutic biopolymers, such as DNA and proteins, to their site of action. Polymers that can respond with a change in conformation to biologically relevant stimuli, such as temperature and pH, are being carefully designed to take advantage of the change in environmental conditions the polymer-drug conjugate encounters upon progression from larger-scale systems in the body to subcellular compartments. Viruses respond to changes in the cellular environment to gain access to their desired region of cells, and much can be learned from the mechanisms they employ in this effort. However, despite the efficiency of therapeutic biopolymers, undesirable immune and inflammatory responses may result from their repeated administration, so synthetic polymers are an attractive alternative. This mini-review examines a range of recently developed variable architecture polymers, mainly focusing on polymers responsive to temperature and pH, covering both synthetic copolymers and derivatives of naturally occurring polymers for advanced drug delivery applications. The polymers discussed in the article have some of the properties that are most important for polymer drug delivery vehicles to be effective, such as biodegradability, specificity, and biocompatibility.

Keywords: Smart polymers, drug delivery, biotherapeutics

Introduction

Delivering drugs to target sites in the body at the right time and in the right dose remains a formidable challenge. This is especially the case for biomacromolecular drugs such as DNA, RNA, short interfering RNA, and therapeutic proteins. Biomacromolecules such as those above injected directly into tissue drain rapidly into the lymphatic system. Furthermore, these complex biopolymers are readily deactivated by enzymes such as DNases and proteases outside their normal biological environment and hence require a carrier vehicle or protective agent when administered as a drug. The delivery vehicles in turn must be able to transport the drug across biological barriers to the target site without causing an unwanted response. The human immune system, for example, has evolved to produce more than 10^8 antibodies and more than 10^{12} different T-cell receptors to destroy foreign material.[1] This means any drug delivery vehicle must evade interaction with a large number of biopolymers in order to be nonimmunogenic. The need for materials that can carry 1 type of biopolymer (the therapeutic) while avoiding interactions with others (plasma proteins, antibodies, non-target-cell membranes) is fueling the development of ever more sophisticated carrier systems. Of particular interest are active or "smart" carrier vehicles, which display 1 set of properties under 1 set of conditions but can change their properties in response to a biological stimulus. For DNA and protein delivery, these properties must include an ability to form stable complexes or conjugates in order to protect the biopolymers from enzymes while overcoming barriers such as cell membranes and, in the case of DNA delivery, the nuclear envelope, combined with the ability to release the biotherapeutic at the target site. Polymers that can vary their architecture from a "closed" to an "open" conformation are perhaps the ideal systems for the contrasting requirements of protection and release. For example, as the polymer and drug cargo travel into different compartments within the body, they can experience a variety of environments. A physical or chemical change in the solvent surrounding the polymer may alter the intermolecular bonding between polymer and solvent, which may also change the affinity of the polymer for the drug. Increased intermolecular bonding with solvent in the case of certain polymers results in a chain-extended conformation with lower affinity for the drug, enabling drug release, whereas increased intramolecular bonds characteristic of a chain-collapsed polymer may form a tighter complex with the drug. As a consequence, polymers can be designed to respond via a conformational change to stimuli in the biological environment, typically by harnessing physiological parameters that are locally regulated, such as temperature and pH, to maintain drug binding in the bloodstream but to effect release intracellularly. In this review we consider several recent developments in the

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exciting field of variable architecture polymers for advanced drug delivery.

Poly(N-Isopropylacrylamide)-Based Temperature-Sensitive Polymers

Poly(N-isopropylacrylamide) (PNIPAm) has been widely investigated for biomedical applications owing to the entropy-driven change of the polymer from a water-soluble coil to a hydrophobic globule at 32°C. This transition, which takes place at the lower critical solution temperature (LCST), can be tuned by variation in co-monomer content so that it is close to normal body temperature. The change in conformation at the LCST can lead to a macroscopic phase separation and precipitation of the polymer from solution or, if the PNIPAm chains are co-polymerized with a nonresponsive component, an altered microscopic architecture of the copolymer. The latter behavior has been harnessed by You and Oupicky to enable biotin ligands to be either freely available in solution below the LCST or sequestered in the center of a micelle above the LCST.[2] Radical addition fragmentation transfer (RAFT) polymerization was used to generate Y-shaped block copolymers of polyethylene glycol (PEG) methyl ether-(mPEG)-co-PNIPAm on a lysine core. Biotin was attached to the free end of PNIPAm, resulting in the heterobifunctional polymers mPEG-Lys-*block*-PNIPAm-biotin (Figure 1 schematic). Heating of these polymers above the LCST of PNIPAm did not cause overall precipitation of the copolymers but instead generated aggregated PNIPAm domains in the form of micelles. The hydrophilic PEG arms surrounded the core and maintained solubility but also masked—or turned off—the biotin ligands, which became hidden in the central core, as demonstrated by a much reduced ability to bind to avidin. Since the biotin-avidin interaction is so widely used in biomedical and diagnostic applications, the ability to selectively turn on and off the biotin signal through polymer phase transitions has many potential practical uses.

The flow and aggregation behavior of PNIPAm-based systems as they undergo coil-to-globule transitions may be of considerable importance for intravenous drug delivery applications. Zhou et al[3] investigated the behavior of PNIPAm microspheres at varying temperatures in a glass pipe with a hydrophilic inner surface at flow rates between 20 and 60 mL/min to simulate laminar blood flow in the microcirculatory system. A temperature gradient was established along the tube such that, as the spheres passed along the tube, an increase in temperature from 23°C to 43°C occurred and the LCST of PNIPAm was crossed. It was found that there was a decrease in microsphere velocity above the phase transition and that aggregation occurred at this point if there was a distance of 6 mm or less between 2 successive microspheres for a flow rate of 20 mL/min. However, when spheres were more than 6 mm apart, aggregation did not occur. In addition, when the number of spheres surpassed a certain critical number (in this case 10), the spheres were immobilized at an aggregation point along the tube corresponding to the phase transition temperature of the PNIPAm coating polymers, causing cessation of flow. Zhou et al proposed that this behavior could be exploited in the circulation by using localized heating to arrest or reduce the flow of the microparticles at a desired location to achieve site-specific drug delivery. At higher flow rates the microspheres would continue to flow in a linear, chainlike procession along the tube. This research, although performed in relation to hydrogel formation, nevertheless provides valuable insight into drug delivery mechanics should these polymers be adopted for an intravenous dosage form.

PNIPAm has been copolymerized with a variety of different polymers to combine thermal response with other favorable properties for drug delivery. An example of this was published by Kim et al, who prepared PNIPAm copolymerized with the biodegradable polymer poly(L-lactic acid) (PLLA), along with a poly(L-lysine) dendron to provide hydrophilicity.[4] Degradation of the PLLA was demonstrated through measurements revealing a notable decrease in viscosity of the linear-dendritic copolymers along with a decrease in molar mass corresponding to loss of the PLLA portion. Fourier transform infrared (FTIR) spectroscopy confirmed hydrolytic cleavage of PLLA ester bonds, and a reduction in degradation rate after 19 days was shown. Degradation of the copolymer was faster at 37°C than at 25°C, indicating the potential utility of this material as a controllable sustained-release device.

pH-Sensitive Gene Delivery Vectors

Oishi et al recently developed a novel copolymer that exhibited a change in conformation in response to a pH stimulus and that also incorporated a targeting ligand.[5] In this case a triblock copolymer composed of lactosylated PEG-*block*-polysilamine-*block*-poly[2-(N,N-dimethylamino)ethyl methacrylate] (lac-PEG-PSAO-PAMA) was complexed with plasmid DNA (pDNA), forming a 3-layered polyplex micelle of nanometer-scale dimensions (Figure 2). When the pH was reduced from 7.4 to 4 the micelles were found to be larger, which was attributed to conformational change of the PSAO regions in the copolymer. As shown in Figure 2, upon protonation of the PSAO chain the polymer became increasingly sterically hindered, changing from an insoluble globular conformation to a rigid expanded rod conformation. The PSAO segment was carefully chosen to incorporate 2 nitrogen centers of differing pK_a values (8.6 and 5.8), thus spanning the pH regimes encountered by the polymeric micelles upon moving from the bloodstream into endosomal compartments,

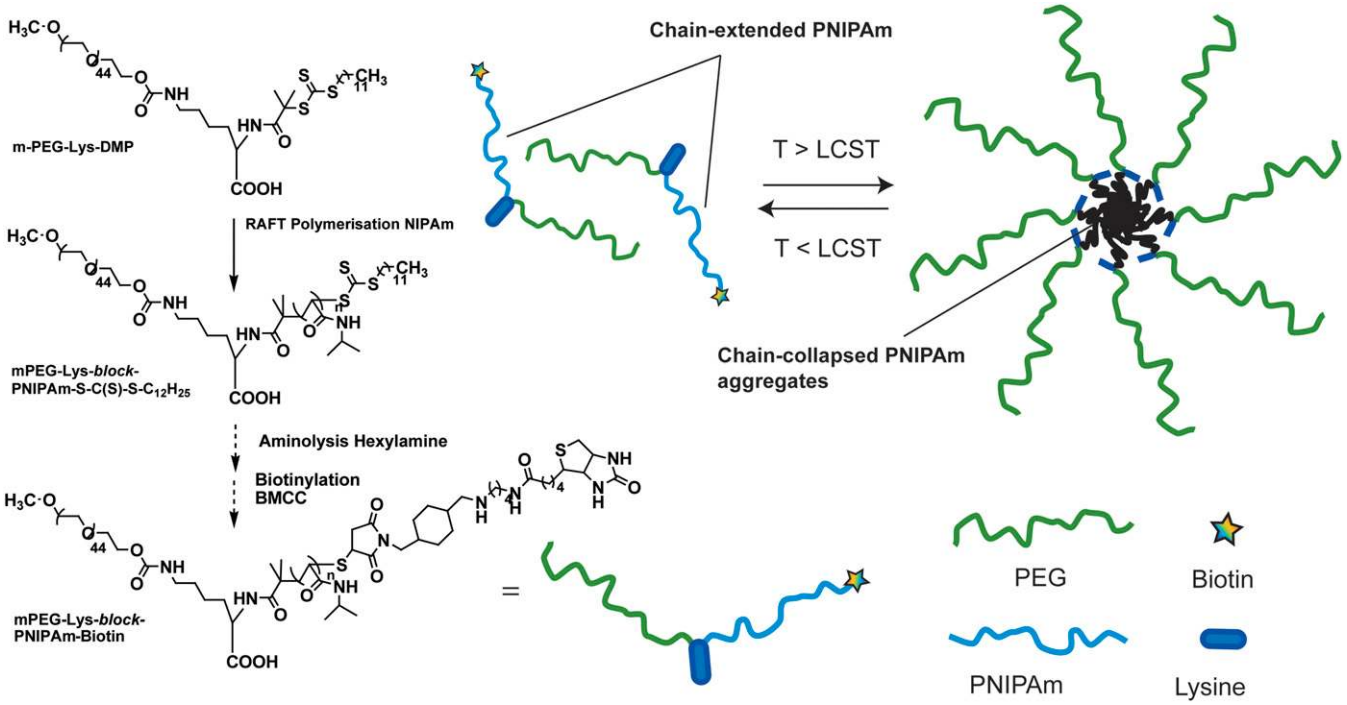

Figure 1. Synthesis of heterobifunctional mPEG-lys-*block*-PNIPAm-biotin block copolymers (left) and schematic representation of the association of mPEG-Lys-*block*-PNIPAm-biotin copolymers in response to temperature (right), with PNIPAm chains collapsing into a micellar core (black central aggregate) as the temperature increases above the LCST, thus hiding the biotin ligands in the center of the aggregate.[2] mPEG indicates poly(ethyleneglycol)-methyl ether; PNIPAm, poly (N-isopropylacrylamide); LCST, lower critical solution temperature; DMP, 2-Dodecylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid; RAFT, Radical Addition Fragmentation Transfer; BMCC, 1-biotinamido-4-[4′-(maleimidomethyl) cyclohexanecarboxamido]butane.

and resulting in a conformational response upon entering the acidic environment of the endosome. This buffering capacity of the polymer through proton uptake was also considered to facilitate disruption of the endosomal compartment, enabling the micelle to escape to the cytoplasm ultimately to facilitate gene expression. Micelles synthesized that were lacking the PSAO responsive unit were less able to transfect cells than were those with PSAO. Also, inhibition of endosomal acidity lowered the transfection efficiency of the lac-PEG-PSAO-PAMA polyplexes. The presence of the lactose group attached to the periphery of the micelle increased transfection efficiency and enabled uptake into hepatocytes through receptor-mediated endocytosis. The polyplex micelles showed more resilience to preincubation with 20% serum, maintaining transfection efficiency, as compared with the control branched polyethyleneimine (PEI)/pDNA polyplexes, which underwent a large reduction in transfection efficiency. Overall, this work highlighted the importance of the endosomal escape mechanism combined with the need for minimal interactions with serum proteins in order to enhance delivery. A detailed examination of the structure-function relationship of each component in the lac-PEG-PSAO-PAMA polyplex micelle was found to give useful insights into the DNA delivery pathway within the cell.

Copolymers of PNIPAm for Gene Delivery

The switchable hydrophilic-to-hydrophobic properties of PNIPAm have attracted attention for nucleic acid delivery. The concept driving this research is that cationic functionalized PNIPAm polymers should be able to compact large, negatively charged nucleic acids above the LCST through charge neutralization combined with hydrophobic interactions to enhance cellular uptake, yet expand below the LCST to allow natural nucleic acid binding proteins to take up the genetic material in the appropriate cellular compartment. Compaction of nucleic acids in this way to generate particles in the 50 to 200 nm range is favorable for delivery in that it enables uptake by endocytotic processes. Initial work on this concept by Hinrichs et al[6] showed that PNIPAm copolymers containing protonated 2-(N,N'-dimethylamino) ethylmethacrylate groups formed complexes with DNA (polyplexes) that varied across LCST and that polyplexes of ~200 nm proved most effective at transfection. Subsequent work by Kurisawa et al established the precedent of varying gene transfection by temperature-induced phase transitions in linear PNIPAm copolymers, with higher protein expression induced by incubation of cells below LCST.[7] More recently, temperature cycling of cells transfected with PNIPAm

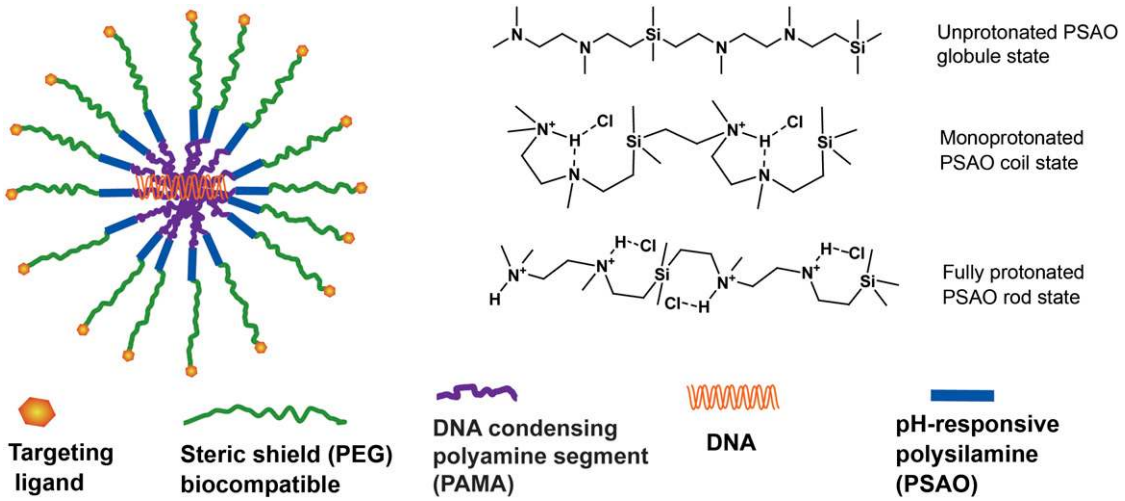

Figure 2. Schematic representation of the 3-layered polyplex micelle made up of ABC triblock copolymers lac-PEG-PSAO-PAMA and DNA (left). The 3 protonation states of PSAO are shown (right), moving from unprotonated PSAO, which is insoluble in water in a globule state (top), to fully protonated PSAO (bottom), which is soluble in water and has a rigid structure with rotation about ethylene bonds restricted by interactions of nitrogen and silicon with protons and chloride ions, respectively.[5] Lac-PEG-PSAO-PAMA indicates lactosylated PEG-*block*-polysilamine-*block*-poly[2-(N,N-dimethylamino)ethyl methacrylate; PEG, polyethylene glycol.

graft-PEI (PNIPAm-g-PEI) polymers across the LCST has been shown to enhance transgene expression.[8-10] Overall transfection efficiencies in these copolymers were comparable or superior to that of PEI, which is widely regarded as the most effective vector for in vitro gene delivery. However, the cytotoxicity of PEI is a significant problem, which has led to PNIPAm copolymers with more biocompatible materials being investigated. A PNIPAm-covinyl laurate copolymer with an LCST of 26°C grafted to chitosan was prepared by Sun et al[11] for gene delivery. It was found that more compact complexes were formed above the LCST of the copolymer when the PNIPAm was in a chain-collapsed conformation and that release of the DNA could be triggered by incubation at 20°C when the PNIPAm chains were expanded and more hydrophilic. Higher transfection efficiencies were reported for polymer-DNA complexes introduced into cells when the culture temperature was reduced to 20°C for 3 hours and then returned to 37°C. However, the detailed mechanisms by which phase transitions in PNIPAm-g-chitosan and PNIPAm-g-PEI copolymers mediate effective gene delivery inside the cell have yet to be resolved.

Non-NIPAm-Based Thermosensitive Polymer Systems

Elastin-like polypeptides (ELPs) are biopolymers that also show a thermoresponsive phase transition that can be adjusted to a biologically relevant temperature. Based upon a hydrophobic domain of human tropoelastin, they consist of pentapeptide repeats of Val-Pro-Gly-Xaa-Gly (Xaa being any amino acid except proline).[12] These polymers show promise as drug delivery/biomedical materials because of the combination of phase transition response coupled with desirable characteristics such as biocompatibility and controlled degradation. ELPs have been produced via recombinant methods from synthetic genes encoding ELP expressed in *Escherichia coli*. Biopolymers produced in this way have the added qualities of precise molecular weights with low polydispersity. A recent study demonstrated the use of ELPs for intra-articular drug delivery.[13] Radiolabeled ELPs designed for aggregation at 37°C displayed half-lives in an injected joint that were 25 times longer than ELPs of similar molecular weight that were soluble and did not aggregate at body temperature. ELPs can also be expressed as conjugated fusion proteins, opening up the possibility of prolonging the half-life of a therapeutic protein in a localized region.[13]

Other examples of variable-architecture thermoresponsive polymer systems that do not rely on PNIPAm include the diblock copolymer methoxy PEG-co-poly(ε-caprolactone) (mPEG-PCL) materials synthesized and characterized by Kim et al.[14] PCL was used because of its biodegradable and biocompatible properties. This copolymer was found to exhibit a sol-gel-sol transition that occurred at a temperature dependent on the PCL length. The phase transition was lowered when the PCL blocks were longer, as expected owing to the hydrophobicity of PCL. As the molecular weight of PCL in the copolymers was varied, keeping mPEG's molecular weight constant at 750, only PCL of molecular weight 2060 and 2440 showed a phase transition at physiological temperature and only in between 15 and 20 wt % solutions.

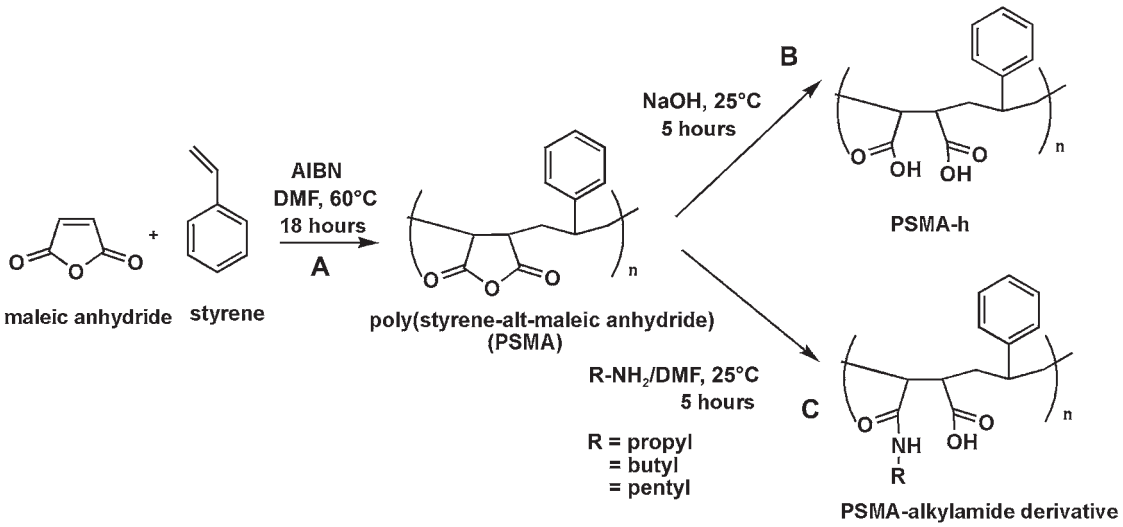

Figure 3. Synthetic scheme for (A) PSMA, (B) hydrolyzed PSMA, and (C) PSMA-alkylamide derivative (adapted from Henry et al[15]). PSMA indicates poly(styrene-*alt*-maleic anhydride); AIBN, azobis(isobutyronitrile); DMF, N,N′ dimethylformamide.

In the gel state the copolymer was found to exist in a fibrillar structure as evidenced by scanning electron microscopy; this macrostructure was postulated to be due to aggregation of the PCL hydrophobic blocks.

pH-Dependent Membrane-Disrupting Polymers for Drug Delivery

An intriguing twist on the use of pH as a trigger to change molecular architecture has been reported by Henry et al.[15] Alkylamide derivatives of poly(styrene-*alt*-maleic anhydride) were prepared such that, instead of swelling through protonation in response to a drop in pH, the polymers changed from being strongly ionized and hydrophilic at pH 7.4 to being hydrophobic at pH values corresponding to those encountered in endosomes. This behavior, analogous to that of conformation-changing membrane active peptides such as melittin and hemagglutinin, was designed to destabilize the endosomal membrane by hydrophobic polymer insertion. The pH response of the polymer arose via carboxylate residues, which were formed by reaction of an alkylamine with the anhydride side chains: ring opening of the anhydride was controlled such that the reaction stopped at amide formation, leaving a residual carboxyl group as well as the alkylamide substituent at each ring-opened anhydride (Figure 3). Following the endosomal pH drop, the carboxylate ions were protonated and the polymer overall became more hydrophobic and fusogenic. Out of the different chain length alkylamines tested, the butylamine and pentylamine derivatives specifically were membrane disruptive in a pH-sensitive manner, being membrane disruptive at pH 5.8 to 6.6 but inactive at pH 7.4. These systems also showed reduced cytotoxicity compared with that of high–molecular weight PEI. The copolymers as designed have a further potential advantage in that the unreacted anhydride groups can be further functionalized, for example, via cleavable linkages to drug compounds, or via permanent linkers to a targeting ligand.

Conclusion

The development of polymers with changing conformations and variable architectures is continuing, and many exciting possibilities are emerging in the pharmaceutical sciences. Research is advancing in the area of controlling drug targeting specificity through creating polymer-ligand conjugates that can be activated to deliver to selected sites via an architecture change when desired. Promising recent examples include double-targeted pH-responsive nanocarriers based on PEG-phosphatidylethanolamine, which present different ligands at pH 7.4 and pH 5 to 6 via hydrazone pH-sensitive linkages.[16] The incorporation of variable architecture polymers into these systems enables selective presentation of targeting ligands. Polymers are also being designed to respond to even more specific localized biological stimuli such as intracellular signaling pathways.[17] Further work to combine the advances in precise synthesis of conformationally switchable and active polymers with site-specific targeting ligands is now beginning to address the most challenging aspects of drug delivery to target sites at the right time, in the right place, and at the right dose.

Acknowledgments

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