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Thermoresponsive polymer-peptide/protein conjugates

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ABSTRACT

This review is focused on recent achievements in the covalent attachment of biological entities, such as oligopeptides, proteins and enzymes, to thermoresponsive synthetic polymers and the influence of conjugation on the properties of macromolecules. Bioconjugation, including polymers, has been intensively studied in the last years. Initially polymer bioconjugates have been developed to be used exclusively for biomedical applications. However, within the last few years, it was shown that this novel class of macromolecules is of high importance in many other rapidly developing areas of material science. Among them, the conjugates of biomolecules with thermoresponsive polymers are of high importance. The presence of biological entities influences the phase transition temperature of the bioconjugates, whereas thermoresponsive chain may increase the activity and solubility of the biological entiety. The ability of the thermoresponsive polymers to self-assemble allows for controlled aggregation of the bioconjugate molecules. Such aggregate formation opens novel routes to the biocarrier of controlled parameter.

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Abbreviations: AAc, acrylic acid; AEMA, *N*-aminoethyl methacrylate; AFM, atom force microscopy; AM, acrylamide; AMA, alkyl methacrylate; AN, acrylonitrile; 3-APMA, 3-aminopropyl methacrylamide; ATRP, atom transfer radical polymerization; AZAA, 4-phenylazophenyl acrylate; AZAAM, *N*-(4-phenylazophenyl) acrylamide; BLG, γ-benzyl L-glutamate; BMA, butyl methacrylate; Boc, *tert*-butyloxycarbonyl; BSA, bovine serum albumin; CTA, chain transfer agent; DEGA-ME, di(ethylene glycol) methyl ether acrylate; DEGMA-ME, di(ethylene glycol) methyl ether methacrylate; DMAM, *N,N*-dimethylacrylamide; DMF, *N,N*-dimethylformamide; DMIAM, 2-(dimethylmaleinimido)-*N*-ethylacrylamide; EGA-ME, ethylene glycol methyl ether acrylate; EMA, ethyl methacrylate; EtOx, 2-ethyl-2-oxazoline; Fmoc, fluorenylmethyloxycarbonyl; GEMA, glucosyoxylethyl methacrylate; GFP, green-fluorescent protein; HEMA, 2-hydroxyethyl methacrylate; HSA, Human Serum Albumin; IgG, immunoglobulin G; IPOx, 2-isopropyl-2-oxazoline; LCST, lower critical solution temperature; MMA, methyl methacrylate; NASI, *N*-acryloxysuccinimide; NCAs, *N*-carboxyanhydrides; NIPAM, *N*-isopropylacrylamide; NMP, nitroxide-mediated radical polymerization; OEGA, oligo(ethylene glycol) acrylate; $OEGA_{454}$, oligo(ethylene glycol) acrylate with average molar mass 454 g/mol; OEGMA, oligo(ethylene glycol) methacrylate; $OEGMA_{360}$, oligo(ethylene glycol) methacrylate with average molar mass 360 g/mol; OEGMA-ME, oligo(ethylene glycol) methyl ether methacrylate; $OEGMA\text{-}ME_{300}$, oligo(ethylene glycol) methyl ether methacrylate with average molar mass 300 g/mol; $OEGMA\text{-}ME_{475}$, oligo(ethylene glycol) methyl ether methacrylate with average molar mass 475 g/mol; PAAc, poly(acrylic acid); PDEAM, poly(*N,N*-diethylacrylamide); P(DEGMA-ME), poly(di(ethylene glycol) methyl ether methacrylate); PEG, poly(ethylene glycol); PEGMA, poly(ethylene glycol) methacrylate; PEGMA-ME, poly(ethylene glycol) methyl ether methacrylate; PEtOx, poly(2-ethyl-2-oxazoline); PIPOx, poly(2-isopropyl-2-oxazoline); PLGA, poly(L-glutamic acid); PNIPAM, poly(*N*-isopropylacrylamide); POEGA, poly(oligo(ethylene glycol) acrylate); POEGMA, poly(oligo(ethylene glycol) methacrylate); P(TEGMA-EE), poly(tri(ethylene glycol) ethyl ether methacrylate); P(OEGMA-ME), poly(oligo(ethylene glycol) methyl ether methacrylate); P(TEGMA-ME), poly(tri(ethylene glycol) methyl ether methacrylate); RAFT, reversible addition-fragmentation by chain transfer polymerization; rh-GH, recombinant human growth hormone; ROP, ring opening polymerization; SEC, size-exclusion chromatography; SET-LRP, single-electron-transfer living radical polymerization; SI-ATRP, surface-initiated atom transfer radical polymerization; SPPS, solid phase peptide synthesis; T_{CP}, cloud point temperature; TCEP, tris(2-carboxyethyl)phosphine; TEGMA-EE, tri(ethylene glycol) ethyl ether methacrylate; TEGMA-ME, tri(ethylene glycol) methyl ether methacrylate; UCST, upper critical solution temperature. For amino acids generally acceptable one letter abbreviations are used.

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1. Introduction

Polymer-peptide/protein conjugates have been of great interest for many years. They are widely studied because of their possible use in medicine, biotechnology and nanotechnology [1]. The high potential of this compound group is illustrated by the number of reviews and papers dedicated to this field [2–8].

Biomedicine, which has begun to dynamically develop in recent years, involves the use of biomolecules, such as proteins, peptides or nucleic acids, for therapeutic or in vivo diagnostic purposes [9,10]. Peptides and proteins have a major role in the treatment of diseases and have fast become an important class of therapeutic agents with the potential to replace many of the existing classic pharmaceuticals. However, the usage of these biomolecules has some limitations, such as very short half-lives in body fluids, fast enzymatic degradation and excretion from the organism [11–18]. These limitations have motivated scientists to improve the stability and prolong the activity of these biomolecules, along with controlled release of the biological species.

The conjugation of proteins [3,19–26] and peptides [20,21,25,27–30] with synthetic polymers endows a number of benefits to the resulting hybrid structures. The bioconjugates can merge the properties of their building components and at the same time, overcome the disadvantages of each of them. For example, when proteins are conjugated, they can maintain or even increase their biological functions, such as enzymatic activity and receptor recognition. Furthermore, the polymer can suppress the surface activity of the protein to prevent its degradation by proteolytic enzymes. The increase in solubility and improvement of the biodistribution of the protein, as a result of its conjugation, are also desirable properties.

An excellent example of improving the pharmacokinetics of a protein by its conjugation to a polymer is the well-known PEGylation, which leads to a significant elongation of the protein's half-life, extending its circulation in the body and increasing its activity [31,32].

The use of stimuli-responsive polymers for the formation of conjugates [18,33–37] receives particular attention because of the similarity of these bioconjugate properties to biomolecules. The response to stimuli is a common process in living systems. Stimuli-responsive polymers respond to different environment stimuli, such as pH or temperature, resulting in reversible changes in their physical or chemical properties [37–41]. Therefore, this class of polymers has been found to be extremely suitable for conjugation with natural molecules.

This review is focused on conjugates containing polymers that respond to temperature. Thermoresponsive polymers have the unique property of undergoing a reversible coil-to-globule transition in response to small changes in temperature [42,43]. Systems involving thermoresponsive polymers are not restricted to aqueous solvent environments, but only aqueous systems are of interest for biomedical applications. Thermoresponsive polymers are soluble in water at low temperatures, but start to collapse at temperatures above the cloud point temperature (T_{CP}) due to dehydration caused by hydrophobic interactions and hydrogen bonding. The minimum on a phase diagram of a polymer solution is called the lower critical solution temperature (LCST). Thermoresponsive polymers form aggregates in dilute solution which are colloidally stable, well-defined and narrowly distributed spherical nanoparticles called "mesoglobules" [44–46]. After cooling below the T_{CP}, the particles dissolve into individual macromolecules.

In this review, the synthetic routes for obtaining thermoresponsive bioconjugates will be discussed. Their properties in solution will be thoroughly examined. The main emphasis of the review will be placed on the behaviour of the bioconjugates of thermoresponsive polymers in solution and on surfaces.

Two groups of thermoresponsive polymers, poly(*N*-isopropylacrylamide) (PNIPAM) and poly(oligo(ethylene glycol) methacrylates) (POEGMAs), are mainly used in conjugations. The most widely studied thermoresponsive polymer, PNIPAM [47], is often applied for conjugation because its LCST is close to the physiological temperature (around 32 °C). The critical solution temperature of conjugates can be controlled by copolymerisation of NIPAM with other monomers. It is well known that the addition of a small amount of hydrophilic component to PNIPAM increases its T_{CP} [2]. In this way, the solution properties of conjugates, and the stability and activity of biomolecules, can be controlled.

POEGMAs have gained considerable interest over the past few years [3,4]. Oligo(ethylene glycol) methacrylates (OEGMAs) are easily polymerisable using techniques such as atom transfer radical polymerisation (ATRP) [5–7] and reversible addition-fragmentation chain transfer polymerisation (RAFT) [8,9]. ATRP and RAFT of OEGMAs allow us to obtain a broad range of linear (homo, random and block), branched, dendritic and star (co)polymers [6,10–12]. In their structure, POEGMAs contain hydrophilic oligo(ethylene glycol) (OEG) side chains and a hydrophobic methacrylate backbone. When the side chains length is shorter than nine ethylene glycol units, the amphiphilic character of the POEGMAs induces their thermoresponsiveness in water solutions. The T_{CP} of POEGMAs in water solutions can be precisely controlled over a wide range of temperatures (20–90 °C) by simply changing the length of the OEG side chains or by copolymerisation of different OEGMA monomers [14,15]. The main advantages of POEGMAs include the lack of T_{CP} hysteresis, the small effects of external factors (e.g. salts and surfactants) on the value of the T_{CP} and a very narrow temperature range for the phase transition [16,17]. As a result of biocompatible OEG pendant groups in the monomers, POEGMAs may be used for biomedical purposes [13].