European Polymer Journal xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Recent developments in polymer–*block*–polypeptide and protein–polymer bioconjugate hybrid materials

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ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form 15 April 2013
Accepted 16 April 2013
Available online xxxx

Keywords:
Controlled radical polymerisation
Block copolymers
Click chemistry
Bioconjugation
Hybrid materials

ABSTRACT

In the last few years, polymer bioconjugates have been shown to be useful in many emerging areas of materials science. Consequently, the synthesis of polymer bioconjugates has suddenly become a central topic in polymer chemistry. The versatility and robust nature of modern synthetic methods such as controlled radical polymerisation (CLRP),[1] ring-opening polymerisation (ROP), and 'click' chemistry make them excellent tools for the preparation of tailor-made polymer bioconjugates. CLRP in combination with other techniques has been shown to be a mature technology for building tailor-made block copolymers and protein–polymer conjugates with a wide range of applications, especially in biomedical domains. This review describes the recent advances and progress in the rapidly expanding field of bioconjugation, outlining the work performed up to 2012.

1. Introduction

Polymer science has developed into a multidisciplinary research area, and now polymers have evolved into superb alternative materials for glass, metal, and wood. In recent decades, polymers have not only been used as industrial bulk materials, but have also attracted much attention in high-technology fields such as nanotechnology, optics, and biomaterials [1]. Therefore, the syntheses of tailor-made macromolecules with desired molecular designs, and consequently, the understanding of their quantitative structure–property relationships have become main focus areas for synthetic chemists.

Polymers can be produced synthetically through various chemical approaches or originate from natural sources. Starch, DNA, and polypeptides are some examples

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[1] **Abbreviations:** AGET, activators generated by electron transfer; AM, amine mechanism; AMM, activated monomer mechanism; ATRP, atom-transfer radical polymerisation; BLG, β-benzyl-L-glutamate; Boc, butylcarbonate; BSA, bovine serum albumin; CLRP, controlled radical polymerisation; CTAs, chain-transfer agents; DCC, dicyclohexylcarbodiimide; DLS, dynamic light scattering; DMAP, dimethylaminopyridine; DSC, differential scanning calorimetry; DMAEMA, dimethylaminoethyl methacrylate; FT-IR, Fourier-transform infrared; GFP, green fluorescent protein; GPC, gel permeation chromatography; HMA, hostasol methacrylate; His, histidine; HO–EbiB, 2-hydroxyethyl 2-bromoisobutyrate; HO–POEOMA, hydroxy-functionalised poly(oligo(ethylene oxide) monomethyl ether methacrylate); HRP, horseradish peroxidase; LCST, lower critical solution temperature; MePEG α-SPA, monomethoxy poly(ethylene glycol)-succinimidyl propionate; MW, molecular weight; n-BuA, butyl acrylate; NAM, N-acryloylmorpholine; NCAs, N-carboxyanhydrides; NHS, N-hydroxysuccinimidyl; NMP, nitroxide-mediated radical polymerisation; NMR, nuclear magnetic resonance; N-TMS, N-trimethylsilyl; PAGE, polyacrylamide gel electrophoresis; PBLA, poly(β-benzyl-L-aspartate); PBLG, poly(γ-benzyl-L-glutamate); PBS, phosphate-buffered saline; PDI, polydispersity index; PDS, pyridyl disulphide; PEG, poly(ethylene glycol); PEGA, poly(ethylene glycol) methyl ether acrylate; PEGMA, poly(ethylene glycol) methylether methacrylate; p(HEMA), poly(2-hydroxyethyl methacrylate); pHPMA, poly(N-(2-hydroxypropyl)methacrylamide); PMA, poly(methyl acrylate); pNIPAM, poly(N-isopropylacrylamide); PS, polystyrene; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; PZLL, poly(ε-carbobenzyloxy-L-lysine); RAFT, reversible addition-fragmentation chain-transfer polymerisation; rh–GH, recombinant human growth hormone; RMA, rhodamine methacrylate; ROP, ring-opening polymerisation; sCT, salmon calcitonin; SDS-PAGE, sodium dodecyl sulphate PAGE; SPPS, solid-phase supported synthesis; t-BA, tert-butyl acrylate; TCEP, tris(2-carboxyethyl)phosphine; THF, tetrahydrofuran; TMB, 3,3′,5,5′-tetramethylbenzidine; VLP, virus-like particle.

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http://dx.doi.org/10.1016/j.eurpolymj.2013.04.017

Please cite this article in press as: Johnson RP et al. Recent developments in polymer–*block*–polypeptide and protein–polymer bioconjugate hybrid materials. Eur Polym J (2013), http://dx.doi.org/10.1016/j.eurpolymj.2013.04.017

of 'natural' polymers, which are all produced by biochemical processes in the living cell. Recently, synthetic polymers have enabled technological advancements that would not have been possible without their additional and valuable functional applications.

Rod–coil block copolymers have attracted significant attention because they can show very different properties in the solution and solid states compared to conventional block copolymers [2–4]. Phase separation in rod–coil block copolymers can occur in the nano-size range instead of on the micro length scales of classical coil–coil systems [5,6]. A specific class of rod–coil block copolymers is formed by those containing an α-helical polypeptide segment rather than the rod segment of most other rod–coil copolymers. In the last five years in particular, the inclusion of peptide segments in block copolymers (molecular chimeras) or bioconjugation via other strategies have been investigated intensively. These biohybrids can open up possibilities for novel materials with enhanced properties in comparison with conventional block copolymers. Most importantly, the polypeptide segments, which are sensitive to environmental changes in, for example, ionic strength, or pH [7], can be utilised in the triggered release of drugs or genetic materials.

Polymer bioconjugates were initially developed by biochemists, and for many years were studied almost exclusively for biomedical applications. However, within the last few years, these novel classes of macromolecules have been shown to be useful in many emerging areas of materials science [8]. Now, the synthesis of polymer bioconjugates has become one of the most important topics in polymer chemistry. For example, modern synthetic methods such as controlled living radical polymerisation (CLRP), ring-opening polymerisation (ROP), 'click' chemistry, and combinations thereof have been proven to be extremely versatile tools for the preparation of tailor-made polymer bioconjugates. Polymer–protein and polymer–peptide conjugates have a relatively long history of therapeutic use, and are already a commercial success in terms of their use in pharmaceuticals following the initial work of Abuchowski and co-workers [9]. Traditionally, poly(ethylene glycol) (PEG) or its derivatives have been used to form new hybrid macromolecules because of their non-toxic, non-immunogenic, and biocompatible properties. However, there has been considerable interest in alternative approaches in order to remove this 'PEG addiction'. Barz et al. [10] reviewed the recent developments in controlled polymerisation techniques that may lead to alternatives to PEG-based systems and can be used to improve the properties of future polymer therapeutics. Polymer bioconjugates with excellent therapeutic potential are also being made with other examples of biocompatible synthetic polymers [11].

The goal of the present review is to provide a description of the recent developments in the emerging research field of bioconjugation. This is not a particularly easy task, owing to the extremely broad categorisation of biological and synthetic polymers. Undoubtedly, the syntheses of polymer bioconjugates are motivated by several structural and functional factors. A simplified view of the fundamental motivations behind bioconjugation is illustrated in Fig. 1, which shows the interaction of various biological systems with manmade polymers in the development of bioconjugate hybrids. Various CLRP methods are extremely versatile tools for preparing tailor-made polymer bioconjugates. Here, we will give an overview of the living radical polymerisation techniques that have been used in the synthesis of biohybrids. Subsequently, the different synthetic strategies for achieving polymer–peptide/protein bioconjugates will also be discussed, with a distinction made between covalent and non-covalent approaches.

2. Controlled/living radical polymerisations

Since 1956, living ionic polymerisations have been of major interest for the synthesis of well-defined polymers. Szwarc reported that in the anionic polymerisations of styrene, the polymer chains grew until all the monomer was consumed, and the chains continued to grow upon further

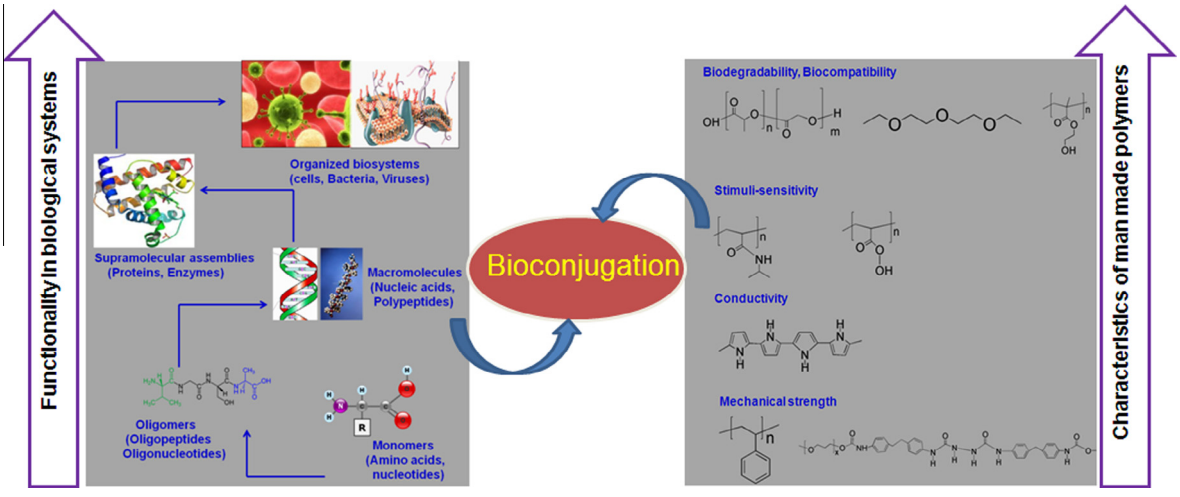

Fig. 1. Schematic representation of the concept of bioconjugation.

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