Progress in Polymer Science 35 (2010) 459–486

Contents lists available at ScienceDirect

Progress in Polymer Science

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

Stability of responsive polymer–protein bioconjugates

Akhilesh Kumar Shakya, Haider Sami, Akshay Srivastava, Ashok Kumar*

Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, 208016 Kanpur, India

ARTICLE INFO

Article history:
Received 7 July 2009
Received in revised form 19 January 2010
Accepted 20 January 2010
Available online 29 January 2010

Keywords:
Responsive polymer
Responsive polymer–protein bioconjugates
End functionalized
Bioconjugation
Stability of responsive polymer–protein bioconjugates

ABSTRACT

Responsive polymer–protein bioconjugates (RPPBs) can be synthesized by different covalent and non-covalent approaches in distinct physical forms (soluble, cross-linked gels, micro/nanoparticles, etc.). These conjugation approaches either involve modification of responsive polymer or protein or both, to achieve higher state of stability. Usually, RPPBs have the potential to be more stable in a reaction environment with broad pH and temperature range without affecting native conformation of the protein. Moreover, they also offer the advantages of reusability, storage and operational stability in contrast to native protein. The stability of RPPB in reaction medium depends on various physiochemical factors such as pH, ionic strength, conjugation approach, ligation chemistry, nature of protein and polymer, etc. Majorly, the stability of RPPB can be enhanced through rigidification of protein structure and creation of controlled microenvironment around the protein, so that native conformation of protein is maintained. This controlled microenvironment may exclude the determinant molecules (e.g., reaction products, inhibitors, etc.) from protein surroundings, therefore minimizing the direct interaction of protein with reaction medium. The present review specifically highlights different ways of RPPB synthesis, types of stabilities of RPPB, factors affecting them and various strategies to enhance the stability. The insight has also been given to details of binding chemistry involved in conjugation of responsive polymer, stability of polyelectrolyte protein complexes and responsive polymer–therapeutic protein bioconjugates.

Contents

Abbreviations: AADG, acrylamide-2-deoxy-D-glucose; AIBN2, 2′-azobisisobutyronitrile; ATRP, atom transfer radical polymerization; CDs, cyclodextrins; CRL, *Candida-rugosa* lipase; CRP, controlled radical polymerization; DMSO, dimethyl sulphoxide; EDCI, 1-ethyl-3-(3-diethylaminopropyl) carbodimide; GEMA, glucosyoxylethylmethacrylate; HEMA, 2-hydroxymethyl methacrylate; LCST, lower critical solution temperature; MPA, methyl phosphonic acid; NAS, *N*-acryloxysuccinimide; NiPAAm, *N*-isopropylacrylamide; NHS, *N*-hydroxysuccinimide; NMP, nitroxide mediated polymerization; ODN, oligodeoxynucleotides; PEG, polyethylene glycol; PEO, polyethylene oxide; PLLA, poly(L-lactic acid); PT, phenothiazine; RAFT, reversible addition–fragmentation transfer polymerization; RPPB, responsive polymer–protein bioconjugates; SEP, synthetic erythropoietin protein; St-HEMA, styrene-2-hydroxyethyl methacrylate.

* Corresponding author. Tel.: +91 512 2594051; fax: +91 512 2594010.
E-mail address: ashokkum@iitk.ac.in (A. Kumar).

0079-6700/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.progpolymsci.2010.01.003

1. Introduction

The cell can acquire unique adaptability in different milieu of living system due to the dynamic nature of biopolymers (e.g., carbohydrates, proteins, nucleic acids, etc.). Among them, proteins are one of the regiospecific macromolecules which have critical importance in the cellular systems. The inherent specificity of the proteins has been utilized for using them as potential therapeutic agents and as molecular sensors in understanding the mechanisms underlying such systems. Because of the easy availability of these naturally occurring and recombinant proteins there is wide interest for their use in various applications. The bioconjugation between protein and polymer with unique properties has opened a new era of biotechnology research. The foremost requirement is to provide stable protein complexes for protein bioconjugation applications. Among the existing bioconjugates a new class of polymer bioconjugates called smart polymer or responsive polymer–protein bioconjugates (RPPB) are being used for various biotechnological applications. The attachment of responsive polymers to protein aims at improving the protein stability and their biochemical/pharmacokinetic profiles.

Over the past two to three decades, researchers have synthesized different responsive polymer–protein bioconjugates by covalent linking of responsive polymers to proteins. Smart properties of synthetic polymers have been exploited for increasing the functional stability of the protein in different reaction microenvironments. In 1970s, pioneering work in polymer–protein bioconjugation was done by Davis and Abuchowski where they observed extended circulation half life time of PEGylated enzyme while retaining the native biological activity in comparison to native enzyme [1]. PEGylation of proteins enhance the plasma half life time of proteins by protecting them from immunological environment of the host system. Followed by this work, Charles et al. [2], reported synthesis of protein polyelectrolyte bioconjugates having multiple carboxyl functional groups (polyelectrolyte block), which are soluble at high pH and insoluble (precipitate) at low pH. Low pH causes protonation of the carboxyl groups, which in effect makes polymer–polymer interactions dominant over polymer–solvent interactions, thus precipitating the bioconjugate. The reversibility of the process at high pH results in solubilization of the bioconjugates. The initial work on responsive polymer protein bioconjugation was done by Hoffman [3], where they successfully conjugated various enzymes and therapeutic proteins to different responsive polymers for industrial and therapeutic applications. The new applications of RPPB cover various areas of biotechnology, specifically in separation of specific cell type [4], downstream processing, clinical diagnostics, immunoassays [5], information storage and retrieval, development of biosensors, biometrics, light harvesting systems, photonics and formation of nanoelectronic devices [6].

The synthesis of RPPB has been carried out by covalent binding of semitelechelic polymers (polymers having one end reactive group per polymer chain) with amino acid residues of protein either randomly or site specifically. Random conjugation of responsive polymer to the protein leads to heterogeneity in the system which causes denaturation of the protein [7]. On the other hand, site directed bioconjugation leads to specific RPPB and have functional stability with better conjugation efficiency than random conjugation. Various synthesis strategies such as direct chemical ligation (where proteins and polymers are covalently attached), macroinitiators approaches (where polymer attached to macroinitiator functionalized protein) have been explored for development of stable site specific RPPB. Synthesis of RPPBs, having uniform molecular weight distribution has been done by employing various living polymerization methods such as nitroxide mediated polymerization (NMP), atom transfer radical polymerization (ATRP) and reversible addition–fragmentation polymerization (RAFT) [8]. In bioconjugation, chemical modification of one of the conjugated partners is required. The precise chemical modification of protein is a tedious task due to its diverse functionalities [9]. Despite the availability of a number of efficient approaches such as site-directed