Colloids and Surfaces A: Physicochem. Eng. Aspects 333 (2009) 82–90

Contents lists available at ScienceDirect

Colloids and Surfaces A: Physicochemical and Engineering Aspects

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

Thermothickening modification of the poly(ethylene glycol) and amino acid ester grafted polyphosphazenes by monomethyl end-capped poly(ethylene glycol) addition

Sungsook Ahn, Sung Won Ahn, Soo-Chang Song*

Life Sciences Division, Korea Institute of Science & Technology (KIST), Seongbuk-gu, Seoul 136-791, South Korea

ARTICLE INFO

Article history:
Received 26 March 2008
Received in revised form 9 September 2008
Accepted 11 September 2008
Available online 2 October 2008

Keywords:
Polyphosphazenes
Poly(ethylene glycol) (PEG)
Graft amphiphilic copolymer
Thermothickening
Hydrogen bonding
Phase transition

ABSTRACT

To control physical properties of the temperature-responsive biomedical materials for targeted applications in easy and safe way biocompatible additives are tried. Systematic controls are studied to obtain effectively controlled results in molecular interaction viewpoint. Thermothickening behaviors of the polyphosphazenes grafted with poly(ethylene glycol) (PEG) and amino acid ester are uniquely modified by physical mixing with another PEG molecules. Depending on the length of the added PEGs and the type of the subtituents of the polyphosphazenes, there are characteristic changes in the maximum viscosity (η_{max}) and the phase transition temperature (T_{max}) reflecting peculiar molecular interactions. For the short chain PEG addition, hydrophobic co-association of the end-capped methyl group of the added PEGs seems to play an important role while for the longer chain PEG additions the interaction between the added and the substituted PEGs seems to be dominating, which changes the η_{max} and T_{max} in a unique way. Longer PEG substituted polyphosphazene is not much affected by the type of the PEGs added probably because of more effective intra-molecular self-interaction than interaction with added ones. For the less hydrophobic amino acid ester substituted polyphosphazene the interaction between the substituted and the added PEGs dominates the de-hydration, which leads to the changes in the phase transition temperature sensitive to the length of the added PEGs. This unique thermothickening modification of the thermosenstitive and biocompatible polyphosphazenes is proposed to be contributed to the special and competitive hydrogen bonding between PEG–water and PEG–PEG molecules, which changes under different conditions like concentration, molecular weight and temperature.

1. Introduction

Poly(ethylene glycol) (PEG) or as it is often called poly(ethylene oxide) (PEO) is a water-soluble synthetic polymer of considerable importance in various applications [1–9]. In tissue engineering applications, PEG has been used as a biocompatible scaffold to improve cell survival and tissue integration [1]. PEGylated proteins are reported to be less-immunogenic and to have increased half-lives in the body [2–4]. PEG has been employed in the design of non-fouling surfaces which provides entropic barrier against other polymers, cells and proteins [5–8]. For the injectable nanoparticulate carriers which are used for site-specific drug delivery or medical imaging, PEG modification provides dramatically increased blood circulation time [9]. This extended circulation time in the body is possible because PEG can bind two to three water molecules per repeat unit which causes the PEGylated compounds to function as though they are five to ten times larger than their true molar mass [8].

In this application point of view, aqueous PEG solutions have drawn a great deal of interest [10–13]. It shows unique phase behaviors where the solubility decreases with temperature. Above the cloud point it undergoes a phase separation but at further higher temperature the homogeneous state becomes stabilized again [14–15]. This generates closed-loop phase region which strongly depends on the molecular weight of the PEGs [10,16]. In terms of the solubility in water, the structure of water molecules surrounding PEGs plays an improtant role where the oxygen–oxygen interdistances of PEG is known to match with the clathrate structure of the pure water [17]. This would cause the principal differences in the solubility of poly(ethylene glycol) (PEG) from those of poly(methylene glycol) (PMG) ($-CH_2O-$) and poly(propylene glycol) (PPG) ($-CH(CH_3)CH_2O-$) which are almost insoluble in water. In terms of its conformation, the hydrophobic $-CH_2CH_2-$ groups are shielded from contacting water while the oxygen is favorable to bind with water. This selective affinity generates optimized conformations tuned to the given condition. At lower temper-

* Corresponding author. Tel.: +82 2 958 5123; fax: +82 2 958 5089.
E-mail address: scsong@kist.re.kr (S.-C. Song).

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doi:10.1016/j.colsurfa.2008.09.045

Table 1
Polyphosphazenes and their physical characteristics used in this study.

Polymer	Structures[a]	T_{max} (°C)[b]	η_{max} (Pa s)[c]	M_p ($\times 10^4$ g mol^{-1})[d]	Conc. (wt%)[e]
1	$[NP(LeuOEt)_{1.39}(AMPEG550)_{0.61}]_n$	35	161.8	3.2	10
2	$[NP(IIeOEt)_{1.05}(AMPEG550)_{0.95}]_n$	48	130.0	3.4	10
3	$[NP(IIeOEt)_{1.20}(AMPEG750)_{0.80}]_n$	48	145.5	3.8	10
4	$[NP(ValOEt)_{1.40}(AMPEG550)_{0.60}]_n$	51	16.75	3.3	10

[a] Substituent ratio was determined by integrating the ^{1}H NMR peak ratio of the representative methyl end protons of each substituents.
[b] Temperature at which viscosity reaches the maximum.
[c] Maximum viscosity was measured at a fixed 0.1 s^{-1} shear rate by 0.5 °C/min heating rate.
[d] Peak position based molecular weight compared with those of standard polystyrene. Gel permeation chromatography (GPC) was measured at 35 °C with 0.8 ml/min flow rate eluted by THF containing 0.1 wt% tetrabutylammonium bromide.
[e] All the polymers were dissolved in de-ionized water with a concentration of 10 wt%.

ature and concentration, PEG shows hydrophilic conformations while at higher temperature and concentration, it turns to be hydrophobic ones. The PEGs in hydrophilic conformations interact more favorably with hydrophilies or polar solvents whereas that in hydrophobic ones with hydrophores or nonpolar solvents [14,18–19]. Meanwhile, in terms of its hydrogen bonding, PEG can dissolve in water with the help of the water layers which surround the PEG mainly by hydrogen bonding between oxygens of PEG and hydrogens of water [16,20]. This hydrogen bonding significantly weakens with temperature increase and also can generate competitive interaction between PEG and water or between PEG and another PEG. However, water is not known to play the role as an inter-molecular bridging between the PEG molecules.

Polymers which exhibit lower critical solution temperature (LCST) are soluble into solvent at low temperature due to effective solvophilic conformation. However, the polymers become insoluble at increased temperature by forming more solvophobic conformation [21–23]. These thermothickening polymers can be usefully modified as injectable delivery carriers where the polymer solutions are easily mixed with drugs at room temperature in either physical or chemical ways and then injected into the targeted body site without any surgery. Due to increased temperature up to body condition, the polymer solution becomes a gel state from which captured bioactive molecules are released in a controlled way [24–25]. In this point of view, hydrophobic and hydrophilic balance is crucial to generate proper thermothickened state while over-hydrophilicity of the polymer cannot induce the phase separation even at higher temperature [26–27]. PEG is one of the useful components which could induce thermothickening when they are properly introduced into the polymer structure due to its effective hydrogen bonding formation with water [10–20]. We have developed our own biodegradable and thermosensitive polyphosphazenes substituted by PEGs and amino acid esters for biomedical applications [28–33]. Polyphosphazene has a unique backbone structure composed of alternating nitrogen and phosphorus. This linkage is highly flexible and easily cleaved by substituted amino acids. Even more, after the degradation only non-toxic final degraded products are left, this is one of the greatest usefulness in biomedical applications [26,28–30]. However, in molecular design point of view, the more useful benefit of the polyphosphazene is the versatility in substitution without changing its backbone length, from which systematic design for the targeted application is more advantageous.

One of the usual methods applied for the study of the lower critical solution temperature (LCST) system would be cloud-point measurement. However, when the phase is separated, the precipitation is dependent on the aggregate size and settling down patterns etc. [34]. Even more, when these polymer solutions are used as delivery carriers in *in vivo* condition, they are not supposed to be under absolute zero shear viscosity. Therefore, slow and homogenous shear force was applied in this study to measure the useful viscosities of the physically mixed systems. Low enough shear rate was applied which makes all the polymer solutions in this study range in the Newtonian flow region so that the measured viscosities can be considered instead of zero shear viscosity to compare the series of polymer solutions. Under this shear rate condition, characteristic maximum viscosity (η_{max}) at a specific temperature (T_{max}) has been obtained for each system, which reflects unique molecular interactions. The polyphosphazenes have been prepared by three different amino acid esters combinated with two different lengths of α-amino-ω-methoxy-poly(ethylene glycols) (AMPEGs) as substituents (Table 1). Poly(ethylene glycol) (PEG) with three different lengths, PEG 350 (number of ethylene oxide, EO ≈ 7), PEG 1100 (EO ≈ 24) and PEG 2000 (EO ≈ 45) have been mixed physically with the aqueous solutions of the aforementioned polyphosphazenes. In this work, thermothickening properties of the biocompatibly designed polyphosphazenes are modified by the addition of the biocompatible PEGs in a reasonably explainable consistency. Competitive and characteristic hydrogen bondings are suggested where the end-group and the molecular weight of the PEGs play an important role together with the polyphosphazene structure. This result sheds light on the controlling methodology over the biocompatible polymeric materials especially focused on the designed physical properties tuned to the targeted applications.

2. Experimental

2.1. Materials

Hexachlorocyclotriphosphazene (Aldrich) was sublimated under reduced pressure at around 55 °C. Anhydrous aluminum chloride (Aldrich) was used without further purification in a dry-box. Leucine ethyl ester (LeuOEt), isoleucine ethyl ester (IleOEt) and valine ethyl ester (ValOEt) were prepared by the literature method [35]. α-amino-ω-methoxy-poly(ethylene glycols) (AMPEGs) with molecular weights of 550 and 750 Da were prepared according to the procedure reported by Loccufier et al. [36]. Tetrahydrofuran (THF) and triethylamine (TEA) (Aldrich) were purified under dry nitrogen atmosphere by refluxing over sodium metal/benzophenone (Acros) and calcium hydride (Acros) respectively. Poly(ethylene glycols) (PEGs) of three molecular weights 350, 1100 and 2000 were used as received (Fluka).

2.2. Instruments and measurements

^{1}H NMR measurements were carried out with a Varian Gemini-300 spectrometer operating at 300 MHz in the Fourier transform mode. Proton decoupled ^{31}P NMR spectra were measured with same spectrometer operating at 121.4 MHz using triphenyl phosphate as an external standard. Waters 1515 gel permeation chromatography was carried out using a Waters 2410 refractive