Macromolecules

Volume 36, Number 1 January 14, 2003

Communications to the Editor

Glucose-Sensitive Aggregates Formed by Poly(ethylene oxide)-*block*-poly(2-glucosyloxyethyl acrylate) with Concanavalin A in Dilute Aqueous Medium

Liang-Chen You, Feng-Zhu Lu, Zi-Chen Li,* Wei Zhang, and Fu-Mian Li

Department of Polymer Science & Engineering, College of Chemistry, Peking University, Beijing 100871, China

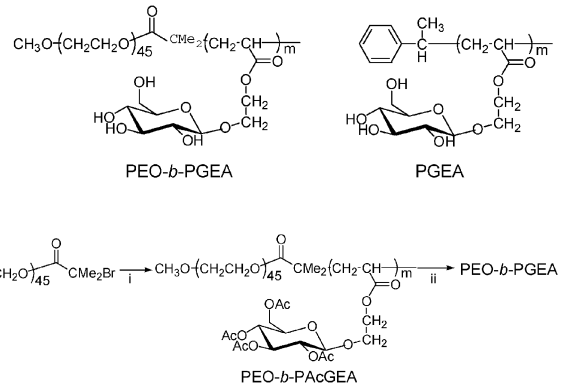
Received September 4, 2002
Revised Manuscript Received November 27, 2002

There is increasing interest in the study of ordered nanoparticles formed by self-assembly of diblock copolymers in solution.[1−5] Among these, micelles from the self-association of amphiphilic diblock copolymers in aqueous media have been extensively studied. Other morphologies of supramolecular assemblies, e.g. vesicles, tubules, etc., have also been reported for a range of amphiphilic diblock copolymers.[6−11] The driving forces for the self-assembly come from both the hydrophobic segments (hydrophobic interaction, segregation) and the repulsive interaction of the hydrophilic segments. Besides amphiphilic diblock copolymers, hydrophilic−hydrophilic diblock copolymers could also form spherical micelle-like assemblies providing that they have specific and strong interactions (electrostatic interaction, hydrogen bonding) between one block.[12−16] If these interactions have been decreased by adding salt or urea or by changing pH, the assemblies may dissociate into single molecules or transformed to other morphologies.[12b,15,16]

Glycopolymer is a kind of water-soluble polymer which shows biocompatible properties and is of biological importance.[17] Because of the effect of concentrated saccharide moieties on the polymer main chain, glycopolymers show much stronger interactions with lectins,

* Corresponding author: Fax 86-10-6275-1708; e-mail zcli@pku.edu.cn.

Scheme 1. Reagents and Conditions: (i) PMDETA, AcGEA, $Cu^{I}Br$, Chlorobenzene, 80 °C; (ii) $CHCl_3$/CH_3OH (9/1, v/v), CH_3ONa, Room Temperature, 30 min, Cation Resin



and this interaction can be disrupted when high concentration of small saccharide existed, thus making it possible to construct glucose-sensitive materials. So far, this concept has been used to fabricate glucose-sensitive hydrogels and other biomaterials.[18] In this communication, we will present a new hydrophilic−hydrophilic diblock copolymer (PEO-*b*-PGEA), which form glucose-sensitive aggregates with Concanavalin A (Con A) in dilute aqueous medium.

PEO-*b*-PGEA was synthesized by atom transfer radical polymerization (ATRP) with the methoxy-end-capped poly(ethylene oxide) macroinitiator as shown in Scheme 1.[19,20] A detailed experimental procedure and characterization of the block copolymers can be found in the Supporting Information. The block copolymer was designated as PEO-*b*-PAcGEA(27) (MWD = 1.12); the numbers in parentheses are the degree of polymerization (DP) of the glycopolymer blocks estimated from ^1H NMR measurements, and the molecular weight of the PEO segment is 2000. After hydrolysis, a double hydrophilic diblock copolymer, PEO-*b*-PGEA(27), was obtained. It should be pointed out that these block copolymers may also be synthesized directly by aqueous

10.1021/ma025641o CCC: $25.00
Published on Web 12/12/2002

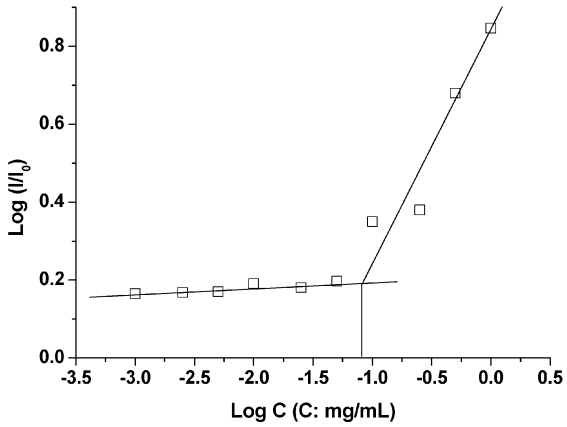


Figure 1. Plots of relative fluorescence intensities (I/I_0) of PNA (2.0×10^{-6} M) as a function of the PEO-*b*-PGEA(27) concentrations at 25 °C ($\lambda_{ex} = 340$ nm).

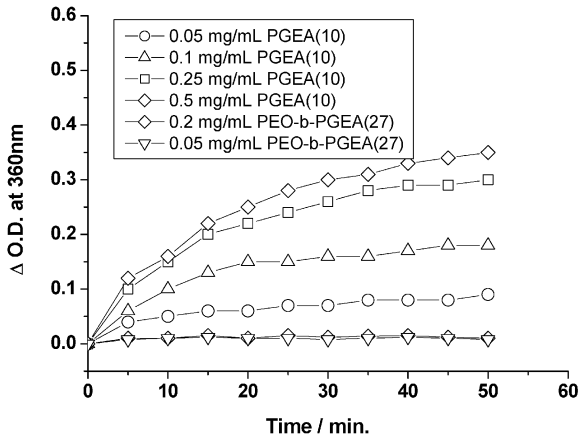


Figure 2. Interaction of Con A (0.83 mg/mL) with PGEA(10) and PEO-*b*-PGEA(27) as measured by transmittance at 360 nm at room temperature.

ATRP recently developed by Armes et al.[21] PAcGEA (DP = 10, MWD = 1.26) was also synthesized by the same procedure with 1-phenylethyl bromide as the initiator through ATRP, and PGEA(10) was obtained after hydrolysis. Recently, there was a good paper about the influence of preparation procedure on glycopolymer composition, in which the author claimed that deacetylation of protected polymer might result in an incomplete deacetylation and yielded products of ill-defined composition.[22] For our polymers, we found very little residual acetyl groups from the ^{1}H NMR spectra, probably due to the relative low molecular weights of polymers. Although the properties of the same polymers prepared directly from the polymerization of deprotected monomer had not been compared, the conclusion of the present paper should not be affected. However, this critical issue is now under investigation by applying the aqueous ATRP of the deprotected monomer.

PGEAs have been synthesized by conventional radical polymerization, and their properties were studied.[23] It has been found that they are soluble in water but tend to aggregate in higher concentrations. The driving force for PGEA association in water may be attributed to the combination of hydrophobic interactions and highly cooperative inter- or/and intramolecular hydrogen bonding. The hydrophobic interaction comes from the polymer backbone, while the hydrogen bonding is mainly from the glucose moieties. Since glucose moieties are densely suspended from the polymer main chain, there might also be very strong intermolecular hydrogen bonding.[24,25] Here, we examined the aggregation behavior of PGEA(10) and PEO-*b*-PGEA(27) in aqueous medium with *N*-phenyl-1-naphthylamine (PNA) as a fluorescence probe. PNA strongly emits in a nonpolar solvent or within a hydrophobic environment, while it is fairly quenched in polar media.[26] This probe has been used to study the aggregation of hydrophobized pulluan in water.[27] When PNA was added to the very diluted aqueous solution of PGEA(10) or PEO-*b*-PGEA(27), very little fluorescence was detected; however, with the increase of polymer concentration, the fluorescence intensity gradually increased, and the emission maximum of PNA blue-shifted simultaneously. The relationship of the relative fluorescence intensity (I/I_0) of PNA as a function of the PEO-*b*-PGEA(27) concentration at 25 °C is shown in Figure 1.[28] It can be seen that the fluorescence intensity values of PNA remained virtually constant below a certain concentration. Above that concentration, the fluorescence intensity increased substantially, reflecting the incorporation of PNA in the hydrophobic region of aggregates. The critical aggregation concentration (cac) was determined by intersecting the two straight lines, and a value of 0.08 mg/mL was obtained for PEO-*b*-PGEA(27). Similar figures for PGEA-(10) were obtained, and its cac was determined to be 0.126 mg/mL. The value for PEO-*b*-PGEA(27) is smaller than that of PGEA(10), which may reflect the effect of molecular weight of PEGA segment; the higher the molecular weight, the smaller the cac, that is to say, PGEA of higher molecular weight tends to aggregate at lower concentration. This was similar as in the case of homopolymers though their molecular weight distributions were broad.[23]

Then we examined the interaction of Con A with PGEA(10) as well as PEO-*b*-PGEA(27) in aqueous solution by measuring the change of optical density at 360 nm. The results are shown in Figure 2. Con A is one of the most widely used lectins in studying the interaction of specific saccharide with protein, particularly for nonreducing D-mannosyl and D-glucosyl residues.[29] The binding of Con A with glycopolymer will result in the precipitation of a Con A-cross-linked aggregates, and the binding constants of glycopolymers to lectins increase with the increase of molecular weight of the glycopolymers.[30] Recently, we found that there also existed reversible specific interaction between Con A and the aggregates formed from PS-*b*-PGEA, whose surface is covered with a glucose group.[31] It can be seen from Figure 2 that the turbidity of the PGEA(10) solution increase with time after addition of Con A, and the increase was related to the polymer concentration. The higher the polymer concentration, the larger the increase of turbidity due to the formation of larger aggregates. Even at a concentration lower than the cac of PGEA(10), for example at 0.05 mg/mL, PGEA solution still became turbid due to the complexation with Con A. The PGEA−Con A complex in the suspension can be dissociated by the addition of free glucose or mannose but not by the addition of free galactose, which is attributable to the weak binding of galactose with Con A.[29d]

In comparison, the behavior of PEO-*b*-PGEA(27) was different than that of PGEA(10). At concentrations higher or lower than cac, no increase of turbidity could be observed for the block copolymer. These results imply that above the cac the block copolymers self-associate to form molecular assemblies with PGEA segments in

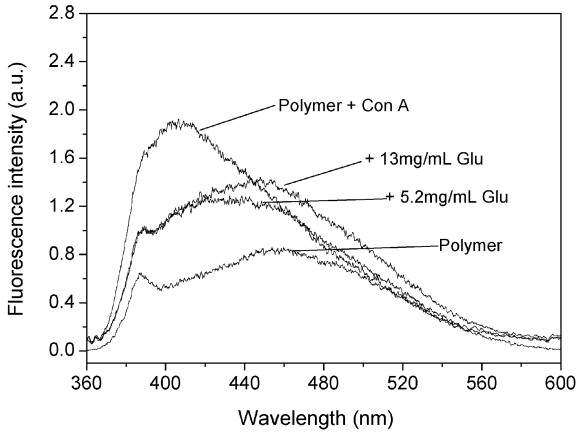


Figure 3. Emission fluorescence of PNA (2.0×10^{-6} M) in PEO-*b*-PGEA(27) (0.05 mg/mL), PEGA-*b*-PGEA(27) (0.05 mg/mL) + Con A (0.15 mg/mL), and after addition of 13 and 5.2 mg/mL of glucose.

the core; thus, their interactions with Con A might be inhibited. While at the concentration lower than cac, the complexation of PGEA segment with Con A was possible and makes the PGEA segment aggregate, but because of the existence of PEO segment, the aggregates are stable in aqueous medium and no increase of turbidity can be observed.

To further verify the above hypothesis that aggregates were formed from the complexation of PGEA segments with Con A, the fluorescence method was again applied. Figure 3 shows the fluorescence spectra of PNA in aqueous solutions of PEO-*b*-PGEA(27) at a concentration of 0.05 mg/mL with or without Con A. As has been discussed above, at this concentration, there is no aggregation of the block copolymers; therefore, the fluorescence intensity of the PNA was low with the peak maximum at 460 nm, which is similar to that of PNA in water. After Con A was added (final concentration of Con A is 0.15 mg/mL), an increase of fluorescence together with a blue shift of emission spectrum was observed. This indicates that PNA was now in an environment of low polarity formed by the complexation of Con A with PGEA segments. When a high concentration of glucose was added to this solution, the maximum emission spectrum was shifted to the longer wavelength, though the intensity did not return to the original value due to the existence of high glucose concentration. This phenomenon can be explained that due to the reversibility of the complexation between PGEA and Con A, even they existed in the interior of the aggregates; glucose could still enter and replace the interaction of PGEA segments with Con A, and thus the aggregates dissociate into single polymer chains, coexisting with the complex of Con A and glucose.

The self-assembly of PEO-*b*-PGEA in aqueous medium is in equilibrium when there is no Con A, which is governed by the copolymer concentration. Even at a concentration larger than cac, copolymer chains (unimers) still exist in the solution. When Con A coexists with the polymer, it will form complex with the PGEA segments, resulting in the decrease of cac and formation of stable aggregates in the case of PEO-*b*-PGEA. This complex cannot be dissociated by just diluting, providing that the copolymer concentration is not too low. This has been confirmed by diluting the aggregate solution prepared from the PEO-*b*-PGEA(27)/Con A mixture at higher copolymer concentration (0.2 mg/mL). Even when

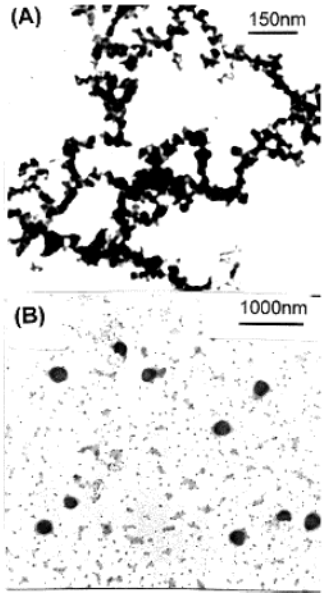


Figure 4. Transmission electron microphotographs of PEO-*b*-PGEA(27) (0.5 mg/mL) and PEO-*b*-PGEA(27) (0.05 mg/mL) + Con A (0.15 mg/mL) in aqueous solution.

the polymer concentration decreased to 0.03 mg/mL, the complex still existed as revealed by fluorescence measurements. Furthermore, aggregate solutions formed by the PEO-*b*-PGEA(27)/Con A mixture or mixture of PEO-*b*-PGEA(27) aggregates with Con A solution at a concentration above the copolymer cac displayed different properties; in the latter case the effect of Con A can only be attributed to its interaction with the unimers existing in the solution, and the PEO chains on the surface of the aggregates may inhibit the complexation of Con A with the PGEA segments in the inner core.

Finally, we examined the morphology of the aggregates with transmission electron microscopy (TEM) formed by PEO-*b*-PGEA(27) in aqueous solution at concentration above cac or below cac but with Con A. The microphotographs are shown in Figure 4. It is clear that at higher concentration the block copolymers self-assemble into aggregates, though the morphologies are not uniform, but small spheres can still be seen, and most of them are interconnected with each other. The average diameters of the spheres are about 10−15 nm. While at lower concentration and in the presence of Con A, larger spherical aggregates were observed, with diameters around 100 nm. The relatively large sizes of these aggregates may suggest that the aggregates are vesicular in nature, which needs further investigation.

In summary, we have shown an example of glucose-sensitive aggregates by the utilization of a glycopolymer-containing diblock water-soluble copolymer and Con A. The reversible formation of aggregates from a dilute solution of this water-soluble diblock copolymer in the presence of Con A and glucose shows potential biomedical applications such as the drug delivery system. However, before this goal can be fulfilled, a lot of work needs to be done; for example, the optimal concentration of Con A and glucose to induce the formation or dissociation of the aggregates should be established. In addition, the time needed for the complete recovery of the single block copolymer chain upon addition of glucose and its relation with copolymer composition, especially the PEO chain length, should also be studied. These questions are now under detailed consideration in our lab.

Acknowledgment. This work was partially supported by the NSFC (#20074002, 29992590-4) and the Foundation for Excellent Young Faculty from the Ministry of Education.

Supporting Information Available: Text giving experimental details on the synthesis of PEO-*b*-PGEA and figures showing their characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

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(28) To get samples for fluorescence measurements, a known amount of PNA in acetone (10^{-3} mol/mL) was added to each of a series of 10.0 mL volumetric flasks, and the acetone was removed. The amount was chosen to give a PNA concentration in the final solution of 2.0×10^{-6} mol dm^{-3}. To each flask was then added a measured amount of PGEA or PEO-*b*-PGEA stock solutions, followed by distilled water. The stoppered flasks were then stirred at room temperature for 3 h to equilibrate the PNA and the aggregates. The samples ranged in polymer concentration from 1.0×10^{-3} to 1.0 mg/mL. Steady-state fluorescence spectra were measured on a Hitachi F-4500 spectrometer with a slit of 5 nm for both excitation and emission. PNA was excited at 340 nm.
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MA025641O