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FOCUS REVIEW

Monodisperse engineered PEGs for bio-related applications

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Abstract

The importance of monodisperse poly(ethylene glycols) (PEGs) and their derivatives is rapidly increasing. Focusing on the merits of heterobifunctional PEGs, we developed a chromatography-free procedure for the preparation of monodisperse monotosylated PEGs. This technique enables the preparation of 8- to 16-mers in relatively large quantities, with good yields and purities, and facilitates the investigation of PEG-based functional molecules. For example, we synthesized structured PEGs consisting of monodisperse PEGs and polyols, which had specific two-dimensional shapes. These structured PEGs exhibited unique properties in aqueous environments; the triangular molecule dehydrated at a lower temperature than linear compounds with a similar molecular weight and could thus suppress the aggregation of proteins at high temperatures. We also found that the introduction of an aromatic group into the PEG skeleton lowered the dehydration temperature, which decreased the critical solution temperatures (LCSTs). Recent achievements in the development of engineered poly(ethylene glycol)s (PEGs) will be highlighted in this review.

Introduction

The importance of water-soluble polymers has been increasing over the last few decades. Poly(ethylene glycol) (PEG) is a typical water-soluble polymer that has been used in a wide variety of products in electronics, cosmetics, and medicines [1]. The important properties of PEGs include their hydrophilicity without ionic functionalities, thermoresponsive hydrophilicity upon temperature change, high affinity for metal cations, and flexibility to avoid the formation of higher-order structures and crystals. PEGs are frequently used as the hydrophilic domains of amphiphilic block copolymers [2]. In bio-related applications, due to their inertness to biomacromolecules, cells, and tissues, PEGs have been used to cover the surfaces of substrates to avoid the adsorption of proteins or the adhesion of cells [3, 4]. In protein chemistry, PEGs have been used to encourage refolding [5], assist in crystallization [6], prolong the blood circulation time [7], and increase the water solubility of proteins and their derivatives. The chemical modification of proteins with PEGs, so-called "PEGylation", is used to improve the efficacy of protein-based drugs, and indeed, several PEGylated drugs have already been commercialized [7]. The hydrophilic and flexible nature of PEGs is advantageous for constructing linkers for antibody-drug conjugates (ADCs) [8].

The synthesis of PEGs was first reported in the middle of the nineteenth century [9]. Since then, various synthetic methods have been developed, most of which include the polymerization of ethylene oxide via acidic, basic or metal catalysis [10]. The resulting PEGs are generated as polydisperse polymers and are given abbreviated names such as PEG8000 in the case of PEG with an average molecular weight of 8 kDa. On the other hand, along with the development of the above-mentioned medicinal applications, the demand for monodisperse PEGs has been growing rapidly, and it is becoming challenging to obtain FDA approval for new materials [7]. Initial efforts to obtain monodisperse PEGs have relied on chromatographic separations, and in fact, many commercially available monodisperse PEGs can be produced using this process. However, while such monodisperse PEGs have narrow molecular-weight distributions, they are still mixtures of different species with very similar molecular weights. Thus, the synthesis of completely pure monodisperse PEGs, sometimes called discrete PEGs, has become an attractive field of study in the last decade [11–17]. Due to the development of new procedures, new molecular designs for water-soluble functional molecules have now become accessible.

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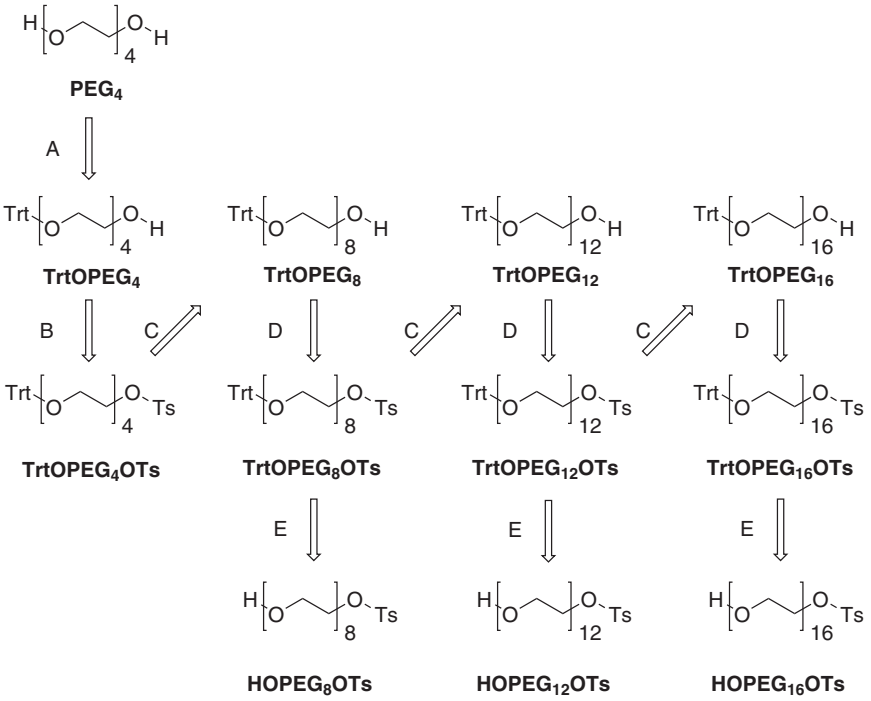


Fig. 1 Reaction schemes for chromatography-free synthesis of $HOPEG_8OTs$, $HOPEG_{12}OTs$, and $HOPEG_{16}OTs$

In this context, we have been involved in the development of functional molecules bearing monodisperse PEGs in their molecular skeletons [18–21]. Recently, we designed a series of water-soluble "engineered PEGs" with various topographies by combining monodisperse PEGs and small molecular units [22–31]. In this Focus Review, the synthesis, properties, and bio-related applications of such engineered PEGs will be highlighted.

Chromatography-free synthesis of heterobifunctional monodisperse oligoethylene glycols

To expand the applicability of monodisperse PEGs, it is important to establish a facile method for their preparation and derivatization on a large scale. For the derivatization of PEGs as hydrophilic units in synthetic and biological molecules or as cross-linkers between such molecules, it is necessary to modify at least one of the two terminal hydroxy groups. Thus, heterobifunctional PEGs (hbPEGs) are advantageous for practical applications. Monofunctionalization of PEGs is the primary method for preparing hbPEGs [32, 33]. However, the efficiency of such desymmetrization reactions is usually low since the reaction process is stochastic. This is a serious disadvantage, particularly for monodisperse PEGs, because except for short PEGs, such as tri- or tetraethylene glycol, they are usually rather expensive starting materials. Thus, the development of efficient synthetic processes for the preparation of hbPEGs is currently of great significance [34–38]. We were inspired to develop an efficient synthetic process to obtain monodisperse hbPEGs in large quantities via simple protocols, in which the final products were monotosylated PEGs that could be derivatized into a wide variety of functionalities for subsequent conjugation with synthetic compounds or biomolecules (Fig. 1) [29, 30].

This procedure started with inexpensive tetraethylene glycol (PEG_4). First, one of two hydroxy groups was protected with a hydrophobic triphenylmethyl (Trt) group. In this step, one equivalent of triphenylmethyl chloride was used to avoid contamination of the product with starting material. The resulting monoprotected compound ($TrtOPEG_4$), containing a small amount of $TrtOPEG_4OTrt$, was coupled with PEG_4 using a Williamson-type ether synthesis, and the remaining hydroxy group was tosylated to give a heterobifunctional octaethylene glycol ($TrtOPEG_8OTs$). Finally, deprotection of the Trt group afforded the desired monotosylated octaethylene glycol ($HOPEG_8OTs$). Further coupling of $TrtOPEG_8OTs$ with tetraethylene glycol (PEG_4) gave longer derivatives via a similar procedure. The important feature of this procedure is that chromatographic purification is not required for any step. The separation of byproducts can be achieved through a simple two-phase extraction in the final step since the possible byproducts are highly soluble in water after deprotection and the monotosylated PEGs are more soluble in organic solvents than the deprotected byproducts. This procedure allows the preparation of $HOPEG_8OTs$, $HOPEG_{12}OTs$, and $HOPEG_{16}OTs$ on multi-gram scales (72, 63, and 62% total yields, respectively) and may be applicable to the synthesis of other monotosylates of different lengths. The purities of $HOPEG_8OTs$, $HOPEG_{12}OTs$, and $HOPEG_{16}OTs$ are 98.7, 98.2,

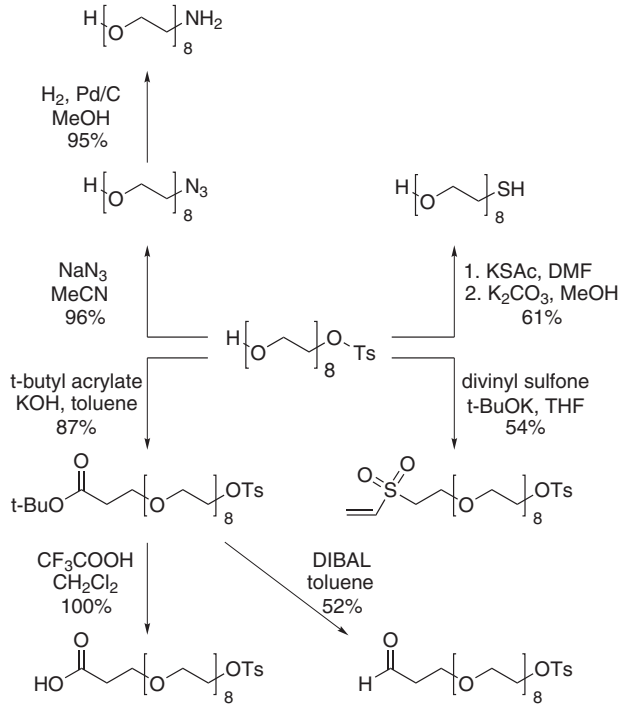


Fig. 2 Chemical conversions of $HOPEG_8OTs$ into synthetically useful compounds

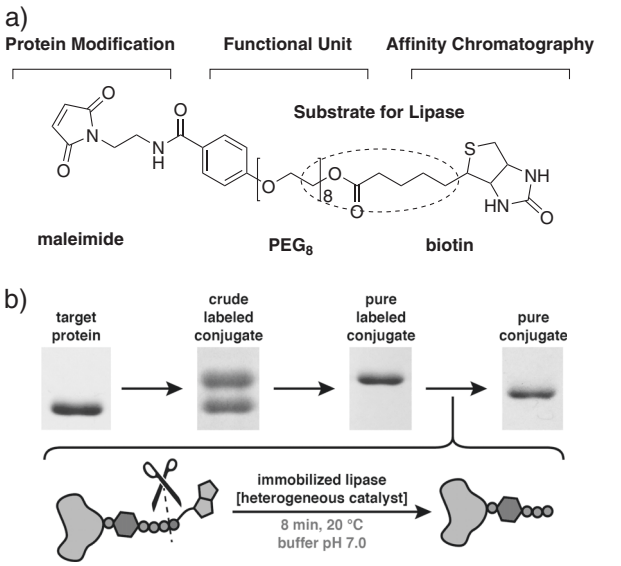


Fig. 3 **a** Structure of mal-PEG-biotin with enzymatically cleavable affinity tag. **b** SDS traces of chemical modification, purification, and cleavage of the biotin tag, as well as reaction of mal-PEG-biotin with β-lactoglobulin as a demonstration of this process. Reprinted with permission from Wawro et al. [31] Copyright 2018 Royal Society of Chemistry

and 97.0%, respectively, comparable to those reported for other procedures using chromatography or commercially available products. These purities are high enough for practical applications. In the synthesis of monodisperse PEGs, the evaluation of purity is crucial. We found that high-performance liquid chromatography (HPLC) with a reverse-phase (RP) column is more suitable than mass spectrometry for this purpose. Additionally, the monotosylate can be converted into biologically useful compounds, such as thiols, amines, and azides, and the hydroxy group can be converted into ester, aldehyde, carboxylic acid and vinyl sulfone moieties in moderate to high yields without affecting the tosylate group (Fig. 2).

Using a short monodisperse PEG, such as octaethylene glycol, we developed a new PEGylation reagent, mal-PEG-biotin, which bears an enzymatically cleavable tag that can be used in purification by affinity chromatography (Fig. 3a) [31]. It is usually difficult to completely purify protein conjugates that are chemically modified with low-molecular-weight synthetic molecules since the molecular-weight difference between the modified and non-modified proteins is not large enough for separation by size-exclusion chromatography. Affinity chromatography affords one solution to this problem; however, the number of available cleavable tags is limited, and some require subjecting the biomolecules to harsh conditions to remove the tag. We took advantage of biotin, which has an aliphatic carboxyl group that can serve as a substrate for lipase after esterification. Thus, one end of mal-PEG_8-biotin bears a maleimide group that can react with the sulfhydryl group of cysteine, and the other end bears a biotin ester moiety that has a high affinity for avidin and is cleavable by lipase. In fact, we succeeded in demonstrating the PEGylation of β-lactoglobulin A (βLgA) using this reagent, which has only one cysteine residue in the peptide sequence. The reaction of mal-PEG_8-biotin with βLgA proceeded smoothly, and the resulting modified βLgA could be purified using monomeric-avidin immobilized on agarose beads (Fig. 3b). The biotin tag could be cleaved by enzymatic hydrolysis using lipase, which was also loaded on the solid support. The lipase-catalyzed cleavage process was rapid: after 6 min, the vast majority of βLgA-1B was consumed, and after 8 min, only hydrolyzed conjugate βLgA-mal-PEG_8OH was detected.

Structured PEGs

We expected that water-soluble molecules, which have specific geometries and dimensions, would possess interesting properties advantageous for the manipulation of biomacromolecules. For PEGs and their derivatives, it is known that the topology of the molecule affects its physicochemical and biochemical properties. For example, branched-type PEG analogs constructed with multi-armed linkers show various micellization behaviors determined by the shape of the molecule [39]. In addition, hyperbranched PEG analogs are reported to have lower viscosities than linear PEGs with similar molecular weights, which is

advantageous for increasing the content of PEGylated proteins for pharmaceutical use [40].

Thus, we decided to develop a new series of water-soluble molecules, termed "structured PEGs", that have various shapes and sizes (unlike conventional linear PEGs) by combining monodisperse PEGs and polyols [22, 28]. PEGs have a hydroxy group at each terminus, which is important for increasing the water solubility of the synthesized molecules. In this molecular design, the use of polyols allows the preservation of hydroxy groups, such as those at the termini of the linear PEGs in the cyclic molecular structure, which increases the hydrophilicity of the compound. Additionally, insertion of the polyol linker helps the PEG chains maintain a more linear geometry in the molecule, unlike cyclic polyethers, such as crown ethers. To demonstrate this hypothesis, we synthesized a series of macrocycles, orthogonal to the hexagonal molecules 2(PEG_4-PE)-6(PEG_4-PE), consisting of different numbers of PEG_4 units connected by pentaerythritol (PE) linkers (Fig. 4). Interestingly, these molecules showed unique properties in aqueous environments, as mentioned below.

First, we investigated the aggregation behavior of these molecules in water at a concentration of 8.67 g/L at 20 °C using dynamic light scattering (DLS) analysis. While 2(PEG_4-PE)-4(PEG_4-PE) did not show aggregate formation under these conditions, 5(PEG_4-PE) and 6(PEG_4-PE) did form aggregates, and 6(PEG_4-PE) formed larger aggregates than 5(PEG_4-PE). This indicates that the hydrophobicity of the molecules increases as their molecular weight increases. A similar trend was also found in the critical aggregation concentration (CAC) of these molecules: 2(PEG_4-PE) showed the highest critical concentration, while 6(PEG_4-PE) showed the lowest. Importantly, in each case, 2(PEG_4-PE)–6(PEG_4-PE) appeared to be more hydrophobic than the corresponding linear PEGs with similar molecular weights. While several properties, such as hydrophobicity, were dependent on the molecular weight, we also found that some properties were independent of the molecular weight, e.g., the thermo-responsiveness of the molecule. PEGs are known to change conformation with temperature [41], and in fact, we also found that 2(PEG_4-PE)-6(PEG_4-PE) showed a similar response when the temperature was increased. However, interestingly, as for aggregate formation, only 3(PEG_4-PE) and 6(PEG_4-PE) exhibited temperature dependencies. In particular, when an aqueous homogenous solution of 3(PEG_4-PE) was heated to 70 °C, submicron-size aggregates were formed. In the case of 6(PEG_4-PE), the size of the aggregates that formed at 20 °C noticeably increased at 70 °C. In contrast, the DLS profiles of 2(PEG_4-PE), 4(PEG_4-PE), and 5(PEG_4-PE) at 70 °C were essentially the same as those at 20 °C. Thus, the aggregation formation behavior of 2(PEG_4-PE)-6(PEG_4-PE) at 70 °C was not dependent on the molecular weight. The dynamic feature of

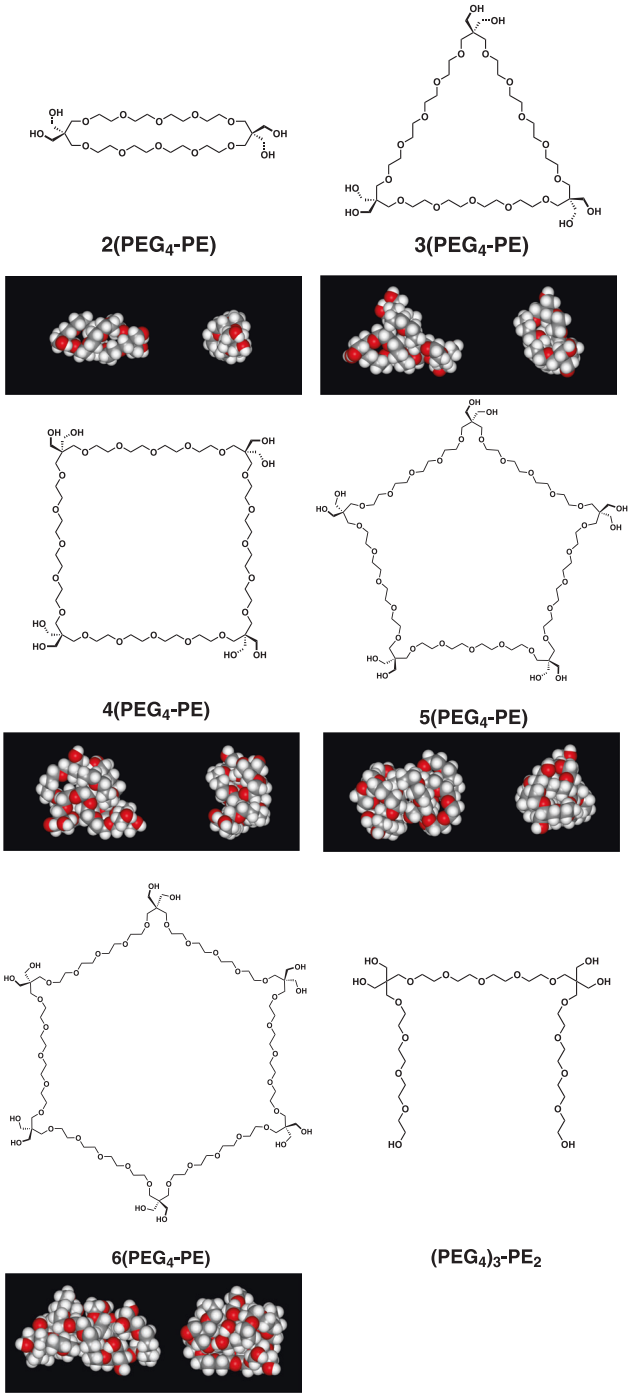


Fig. 4 Structures and molecular modeling results of structured PEGs: 2(PEG_4-PE)-6(PEG_4-PE) and $(PEG_4)_3$-PE_2 consisting of PEG_4 and pentaerythritol (PE)

these molecules, as observed in their NMR spin-lattice relaxation times, also showed a rather weak dependence on the molecular weight. Indeed, at 30 °C, the dynamics of 6(PEG_4-PE) were the most restricted, despite having the largest ring size, while 4(PEG_4-PE) and 5(PEG_4-PE) showed less restricted dynamics than 6(PEG_4-PE), but with a similar degree of restriction to each other. Overall, 2

(PEG_4-PE) showed the fewest restrictions. However, at 70 °C, 3(PEG_4-PE) showed the largest change in dynamics and became the least restricted among the synthesized materials. These varied dynamic features and temperature responses are correlated to the thermal responses of the aggregation formation behavior mentioned above. The other size-independent property is the minimum area/molecule A_{min} (nm^2) evaluated based on concentration-dependent surface tension changes, and 3(PEG_4-PE) and 4(PEG_4-PE) showed similar values, as did 5(PEG_4-PE) and 6(PEG_4-PE) (Fig. 4). This feature is supported by molecular modeling studies of these molecules, which showed that the molecules have rod-like conformations for 2(PEG_4-PE), plate-like conformations for 3(PEG_4-PE) and 4(PEG_4-PE) and spherical conformations for 5(PEG_4-PE) and 6(PEG_4-PE). These results strongly indicate that the topologies of these structured PEGs significantly impact their physicochemical properties in aqueous environments, which makes topology an important factor in the design of water-soluble molecules for bio-related applications.

As an application of these structured PEGs, we recently found that 3(PEG_4-PE) can significantly suppress the thermal aggregation of proteins, such as lysozyme [22]. Detailed studies on the thermal response of 3(PEG_4-PE) showed that in water at approximately 60 °C, this molecule undergoes dehydration, accompanied by a *gauche*-to-*trans* conformational change in the PEG_4 chains. In contrast, in the case of $(PEG_4)_3$-PE_2, dehydration occurs above 90 °C, at a temperature similar to that for conventional linear PEGs. This means that the two-dimensional shape of 3(PEG_4-PE) decreases the dehydration temperature. The dehydration is consistent with the DLS measurements at 70 °C, which showed the formation of large aggregates that were not observed at 20 °C. This suggests that 3(PEG_4-PE) becomes more hydrophobic at 70 °C than at 20 °C, and the switch in hydrophilicity/hydrophobicity occurs at approximately 60 °C.

This hydrophilicity/hydrophobicity switching of 3(PEG_4-PE) at 60 °C inspired us to use 3(PEG_4-PE) to suppress the thermal aggregation of proteins. Upon heating, most proteins undergo thermal denaturation, which eventually causes the formation of insoluble precipitates. This is because after denaturation, the hydrophobic residues, which are usually located inside the folded protein, are exposed to the aqueous environment. This process is usually irreversible, and in most cases, proteins lose their activities. We considered that 3(PEG_4-PE), which is more hydrophobic following dehydration above 60 °C, might interact with the hydrophobic surface of the denatured protein, thereby suppressing the formation of aggregates. Indeed, we found that 3(PEG_4-PE) suppressed the thermal aggregation of lysozyme quite efficiently (Fig. 5a). Even at a concentration of 5 mg/mL, a much lower concentration than required for arginine (a common aggregation suppressor for proteins),

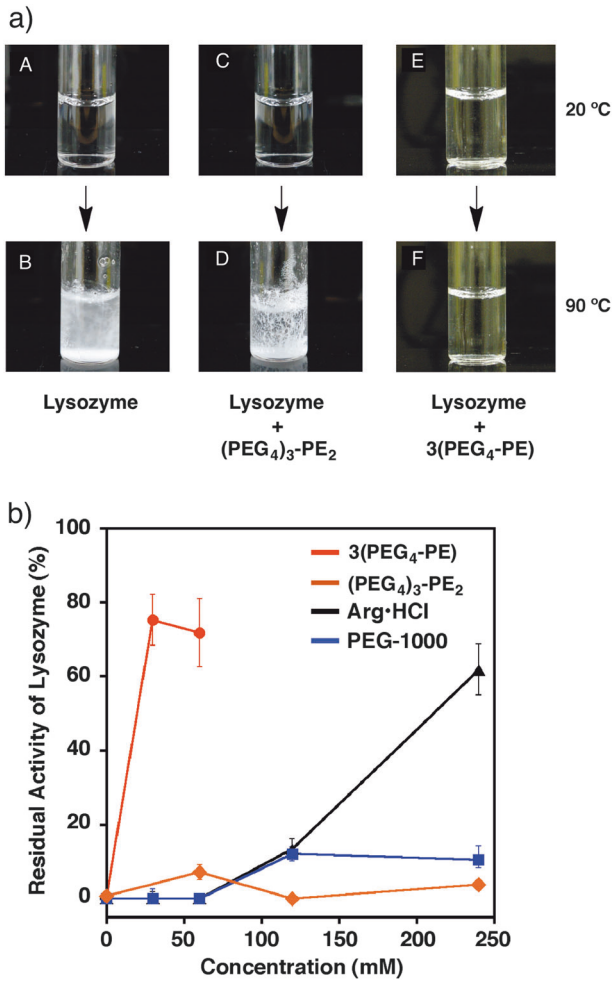


Fig. 5 Suppression of lysozyme aggregation at 90 °C. **a** Photographs of lysozyme in PBS (3.0 mg/mL) at 20 °C (upper) and 90 °C (lower). (A and B) No additive, (C and D) with $(PEG_4)_3$-PE_2 (30 mg/mL), and (E and F) with 3(PEG_4-PE) (30 mg/mL). **b** Residual enzymatic activity of lysozyme (3.0 mg/mL = 0.21 mM) after heat treatment (98 °C, 30 min) with various concentrations of additives (red circles: 3(PEG_4-PE), orange diamonds: $(PEG_4)_3$-PE_2, black triangles: L-arginine hydrochloride, and blue squares: PEG-1000). Adapted with permission from Muraoka et al. [22] Copyright 2013 John Wiley and Sons (Color figure online)

60% of the enzymatic activity of lysozyme was recovered in the presence of 3(PEG_4-PE) after heating to 90 °C and cooling to room temperature (Fig. 5b). On the other hand, the use of $(PEG4)_3$-PE_2, which is linear, resulted in precipitates upon heating, and hardly any enzymatic activity was recovered. ^{1}H NMR and circular dichroism (CD) spectroscopic studies of a mixture of 3(PEG_4-PE) and lysozyme revealed that 3(PEG_4-PE) does not stabilize the higher-order structure of lysozyme at high temperatures; instead, it suppresses aggregation of the denatured lysozyme. Interestingly, a similar stabilization effect was also observed for other proteins, such as carbonic anhydrase and phospholipase [23]. Notably, the aggregation-suppression effect of 3(PEG_4-PE) is observed only for proteins that denature above 60 °C, indicating that the dehydration

temperature of 3(PEG_4-PE) is crucial for its ability to suppress the aggregation of proteins.

Engineered PEG derivatives bearing aromatic groups

The above-mentioned structured PEGs consisted only of oligoethylene glycol and PE units. However, the macrocyclic structures of these compounds likely increase the hydrophobicity of the molecules, resulting in their unique properties. This finding prompted us to develop PEG-based amphiphilic compounds with a small proportion of aromatic groups in the molecular structures.

We introduced a phenyl group at one end of monodisperse tetraethylene glycol (PEG_4) and octaethylene glycol (PEG_8) and compared their thermoresponsive behaviors in water (Fig. 6a) [25]. Indeed, both $PhOPEG_4$ and $PhOPEG_8$ formed aggregates in water, and the CACs of $PhOPEG_4$ and $PhOPEG_8$ were found to be 0.80 and 1.3 mM, respectively, based on their ^{1}H NMR chemical shifts. Interestingly, upon elevation of the temperature, the size of the aggregates of $PhOPEG_4$ decreased, while those formed by $PhOPEG_8$ increased. In particular, the thermal response of $PhOPEG_4$ was similar to those of ionic surfactants, while that of $PhOPEG_8$ was more polymer-like. Thus, the chain length of the oligoethylene glycol units significantly influenced the thermoresponsive properties of these materials. A difference in the thermal responses of $PhOPEG_4$ and $PhOPEG_8$ was also found in terms of their dehydration temperatures. Non-modified PEG_8 does not dehydrate below 80 °C, while $PhOPEG_8$ dehydrates at approximately 50 °C. In the case of $PhOPEG_4$, it is likely that dehydration occurs at approximately 40 °C, but the transition is not as clear as it is for $PhOPEG_8$. These results indicate that the introduction of a phenyl group at one terminus decreased the dehydration temperature, and the length of the PEG chain significantly influenced both the transition temperatures and aggregate formation behaviors. A detailed ^{1}H NMR analysis of the conformation of the PEG chains suggested that the *gauche* form is favored at the carbon−carbon bond of the ethylene oxide group adjacent to the phenyl group. This tendency was more clearly observed for $PhOPEG_8$ than for $PhOPEG_4$.

Usually, short PEGs with molecular weights less than 1 kDa cannot efficiently suppress the thermal aggregation of proteins. In sharp contrast, we found that $PhOPEG_8$ exhibited a moderate aggregation-suppression capability for lysozyme at high temperature [27]. This stabilization effect was not observed for $PhOPEG_4$ or PEG_8 (Fig. 6b). In this case, the suppression efficiency was concentration dependent. In particular, when the concentration of $PhOPEG_8$ was 1.0 mM, lysozyme precipitated upon heating at 90 °C, while the formation of precipitates was suppressed in the presence

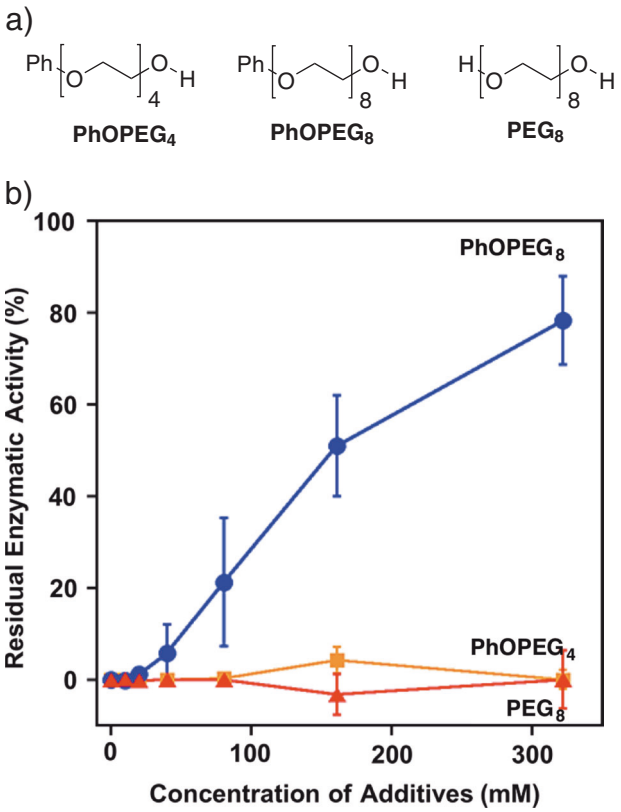
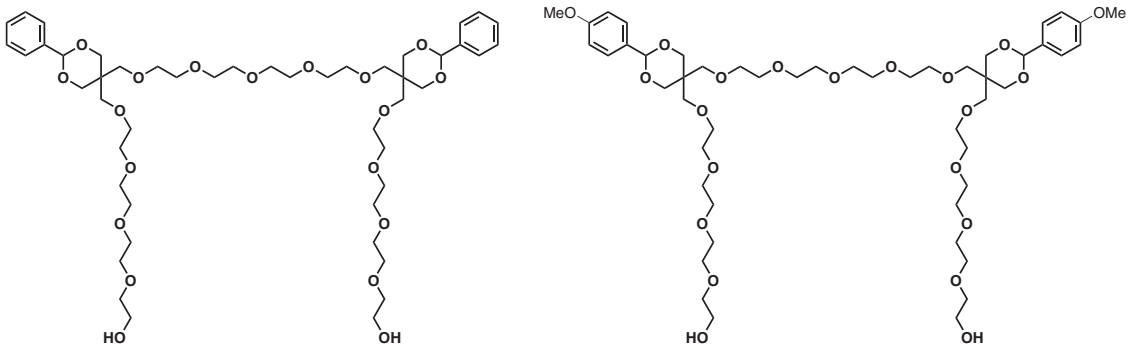


Fig. 6 **a** Structures of $PhOPEG_4$, $PhOPEG_8$, and PEG_8. **b** Residual enzymatic activities of recovered chicken egg white lysozyme (0.037 mM) in PBS buffer (pH 7.4) after heat treatment at 90 °C for 30 min with various concentrations of PEG_8 (red triangles and solid line), $PhOPEG_8$ (blue circles and solid line), and $PhOPEG_4$ (orange squares and solid line). Adapted with permission from Sadhukhan et al. [27] Copyright 2015 Royal Society of Chemistry (Color figure online)

of 1.6 mM $PhOPEG_8$. In this case, since the CAC of $PhOPEG_8$ is 1.3 mM in water, it is likely that the aggregation of $PhOPEG_8$ is essential for suppressing the aggregation of lysozyme. Another interesting feature of $PhOPEG_8$ is that it can assist in the refolding of chemically unfolded lysozyme. At a high concentration of $PhOPEG_8$, such as 480 mM, 38% of the enzymatic activity of lysozyme was recovered, while PEG_8 and $PhOPEG_4$ provided only 0.47 and 1.0% recovery, respectively. This chaperone-like property has not been observed for previously reported structured PEGs.

We also prepared larger molecular systems, $(PEG_4)_3PE_2$-Ph_2 and $(PEG_4)_3PE_2$-$pMeOPh_2$ (Fig. 7), consisting of three PEG_4 and two PE units connected in a linear manner. The aromatic units were introduced into the PE units in the middle of the main chain [26]. We found that this second series of compounds exhibited lower critical solution temperatures (LCSTs), 24.6 and 26.4 °C, respectively, which led to aqueous two-phase separation. LCSTs are usually observed for water-soluble polymers. While the corresponding linear molecule, which has no aromatic groups, does not show an LCST, the introduction of an aromatic



$(PEG_4)_3PE_2$-Ph_2 $(PEG_4)_3PE_2$-$pMeOPh_2$

Fig. 7 Structures of $(PEG_4)_3PE_2$-Ph_2 and $(PEG_4)_3PE_2$-$pMeOPh_2$

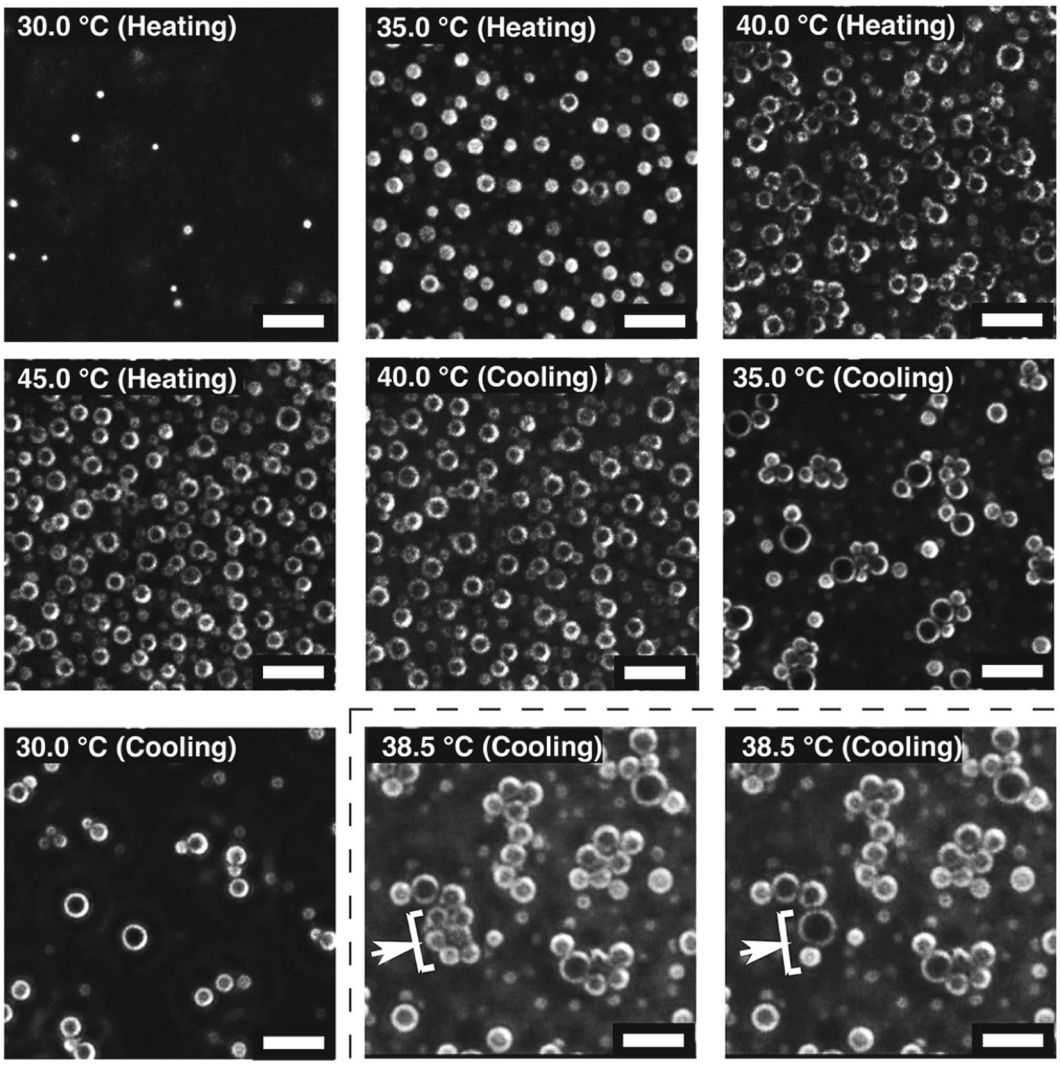


Fig. 8 Phase-contrast micrographs of $(PEG_4)_3PE_2$-Ph_2 in water (20 mM) upon temperature elevation and reduction (20–45 °C) at a rate of 1.0 °C/min. White arrows and square brackets at 38.5 °C in the cooling step indicate fusing of spherical objects. Scale bars: 20 μm. Reprinted with permission from Kawasaki et al. [26] Copyright 2014 John Wiley and Sons

group allowed these non-polymeric molecules to behave like polymers. Interestingly, the temperature-dependent transmittance changes in these molecules revealed hysteretic behavior. These transmittance changes were dependent on the concentrations of $(PEG_4)_3PE_2$-Ph_2 and $(PEG_4)_3PE_2$-$pMeOPh_2$, and at concentrations above 5.0 mM, the T_{50} (the temperature at 50% transmittance) in the cooling process was lower than that in the heating process. This behavior switched at concentrations below 5.0 mM. Meanwhile, the heating and cooling curves mostly overlapped at 5.0 mM. Hysteretic behaviors were also observed in the DLS profiles, which showed a concentration dependence consistent with the transmittance changes mentioned above. In each case, larger aggregates were formed at higher temperatures. Phase-contrast microscopic observations for these compounds at 20 mM clearly showed the formation of μm-sized aggregates upon temperature elevation (Fig. 8). While the size of the aggregates seemed constant in the heating process, the aggregates began to merge and form larger aggregates during the cooling process. This may be a major reason for the hysteretic behavior observed in the transmittance measurements. The thermal responses of $(PEG_4)_3PE_2$-Ph_2 and $(PEG_4)_3PE_2$-$pMeOPh_2$ as investigated by ^1H and ^{13}C NMR spectroscopy suggested that upon temperature elevation, these molecules undergo *gauche*-to-*trans* conformational changes in the PEG chains. In addition, the signals corresponding to the aromatic groups shifted upfield, suggesting aggregation of the aromatic moieties at high temperatures. Thus, the increases in the size of the aggregates upon temperature elevation was likely due to the enhanced hydrophobicity of the PEG chain induced by the conformational change and inter-aromatic interactions introduced in the PEG chain.

The aqueous two-phase separation of $(PEG_4)_3PE_2$-Ph_2 and $(PEG_4)_3PE_2$-$pMeOPh_2$ occurs near their systemic temperatures. This is advantageous for biological applications, and indeed, we have demonstrated the extraction of peptides using this phenomenon. Trypsin-digested bovine serum albumin (BSA) was extracted with $(PEG_4)_3PE_2$-Ph_2 and $(PEG_4)_3PE_2$-$pMeOPh_2$ at 40 °C and separated from the aqueous phase. MALDI-TOF MAS analysis of the extracts revealed that among the six peptides present in the digested BSA, three were selectively extracted into these amphiphilic phases. The extracted peptides were rather hydrophobic and included relatively high contents of aromatic residues. Since fine-tuning of the molecular structure is possible in this case, it should be possible to develop more selective systems that use aqueous two-phase separation.

Outlook

Our chromatography-free process for the synthesis of monodisperse PEGs has significantly improved access to these compounds, which can serve as components for synthetic and biological functional molecules. Our findings suggest that the molecular topology and weak amphiphilicity, unlike those of well-established strong detergents, are important for the tuning and manipulation of the properties of biological molecules. For example, the introduction of a small proportion of aromatic groups into the molecular design of the PEG-based skeleton would be an efficient strategy for developing reagents that can suppress aggregation and/or assist in the refolding of proteins. Further development of molecular designs is currently underway. In addition, investigating the difference in the properties of poly- and monodisperse PEG derivatives for bio-related applications is also of great interest. This would be particularly important for short oligomers with molecular weights below approximately 2 kDa.

Compliance with ethical standards

Conflict of interest The author declares that he has no conflict of interest.

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