RESEARCH ARTICLE

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Membrane Anchored Polymers Modulate Amyloid Fibrillation

*Newton Sen, Gerd Hause, and Wolfgang H. Binder**

The nucleating role of cellular membrane components, such as lipid moieties on amyloid beta (Aβ_{1-40}) fibrillation, has been reported in recent years. The influence of conjugates fabricated from lipid anchors (cholesterol, diacylglycerol) and hydrophilic polymers on Aβ_{1-40} fibrillation is reported here, aiming to understand the impact of polymers cloud point temperature (T_{cp}) and its hydrophobic tails on the amyloid fibrillation. Novel lipid-polymer conjugates, consisting of poly(oligo(ethylene glycol)$_m$acrylates) and hydrophobic groups (diacylglyceryl-, cholesteryl-, octyl-, decyl-, hexadecyl-) as anchors are synthesized using reversible addition-fragmentation chain transfer (RAFT) polymerization, allowing to tune the hydrophilic-hydrophobic profile of the conjugates by varying both, the degree of polymerization (*n*) and number of ethylene glycol units (*m*) in their side chain. The impact of these conjugates on Aβ_{1-40} fibrillation is investigated via in vitro kinetic studies and transmission electron microscopy (TEM). Hydrophobic lipid-anchors are significantly delaying fibrillation (both lag- and half times), observing similar fibrillar structures via TEM when compared to native Aβ_{1-40}. Other hydrophobic end groups are also delaying fibrillation of Aβ_{1-40}, irrespective of their "*n*" and "*m*," whereas more hydrophilic polymers (both with longer ethylene glycol-sidechains, $m = 3$ for octyl, decyl and $m = 5$ for cholesterol) are only marginally inhibited fibrillation.

1. Introduction

The solubility of proteins is fundamental to protein homeostasis and functionality.[1] Nucleation-driven polymerization of soluble proteins leads to growth and subsequent senile mature aggregates, comprised of sterically zippered perpendicular cross-β sheets, both either functional and pathological in nature.[2] Such nucleation induced dynamic self-assembly polymerization processes of soluble proteins and peptides into insoluble aggregates are responsible for numerous debilitating medical conditions ranging from aging-associated neurodegenerative pathological disorders like Alzheimer's disease, Parkinson's disease to lifestyle changing disorders like type II diabetes,[3,4] whereas other functional amyloids are responsible for biological functions like storage of hormones in the endocrine system.[5] Several small molecules and proteins have been explored to interfere with fibrillation by stabilizing early aggregation states to breaking of senile amyloid plaques in vitro. Thus, proteins,[6] peptides and peptidomimetics,[7–10] glycopeptidomimetics,[11] macrocyclic amyloid β-sheet mimics (ABSMs)),[12] photooxygenation catalysts,[13] nanomaterials,[14] metal chelation,[15] small-molecule compounds,[16] and polymer-peptide conjugates[17] have been proven to intervene with amyloid Aβ fibrillation in vitro.

The supramolecular self-assembly of pathogenic amyloid beta (Aβ) peptides (both Aβ_{1-40} and Aβ_{1-42}) originates from transmembrane amyloid precursor proteins (APP) by forming initial micelle like soluble oligomeric aggregates and finally assembling into amyloid fibrils.[18] Thus, interactions between Aβ peptides and cell membranes play a major role in Aβ pathogenicity, where interfaces act as loci for oligomers and are proposed to promote nucleation induced fibrillation.[19,20] Membrane interactions are the basis of varying concentrations between extracellular and intracellular regions,[19,21] due to pore formation, detergent effects and adsorption onto the membrane interface, finally inducing disruption of the cell membrane integrity.[22,23] As cell membranes are containing cholesterol and diacylglycerol phospholipids, the balancing and counterbalancing of inhibitory or acceleratory[21,24–26] and chaperone-like activities[26] have been investigated so far using phospholipid vesicles in view on their lipid bilayer composition, thickness, curvature and lipid-to-peptide ratio.[21,22,24–30] Some mechanistic understanding of a few membrane lipids on amyloid aggregation has also been deciphered, pointing to a heterogeneous primary nucleation by cholesterol[31,32] and secondary nucleation by seeding (e.g., by ganglioside-lipids).[24]

N. Sen, Prof. W. H. Binder
Chair of Macromolecular Chemistry
Faculty of Natural Science II
Von-Danckelmann-Platz 4
Institute of Chemistry
Martin-Luther University Halle-Wittenberg
Halle (Saale) D-06120, Germany
E-mail: wolfgang.binder@chemie.uni-halle.de

Dr. G. Hause
Biocenter
Martin-Luther University Halle-Wittenberg
Weinbergweg 22, Halle (Saale) D-06120, Germany

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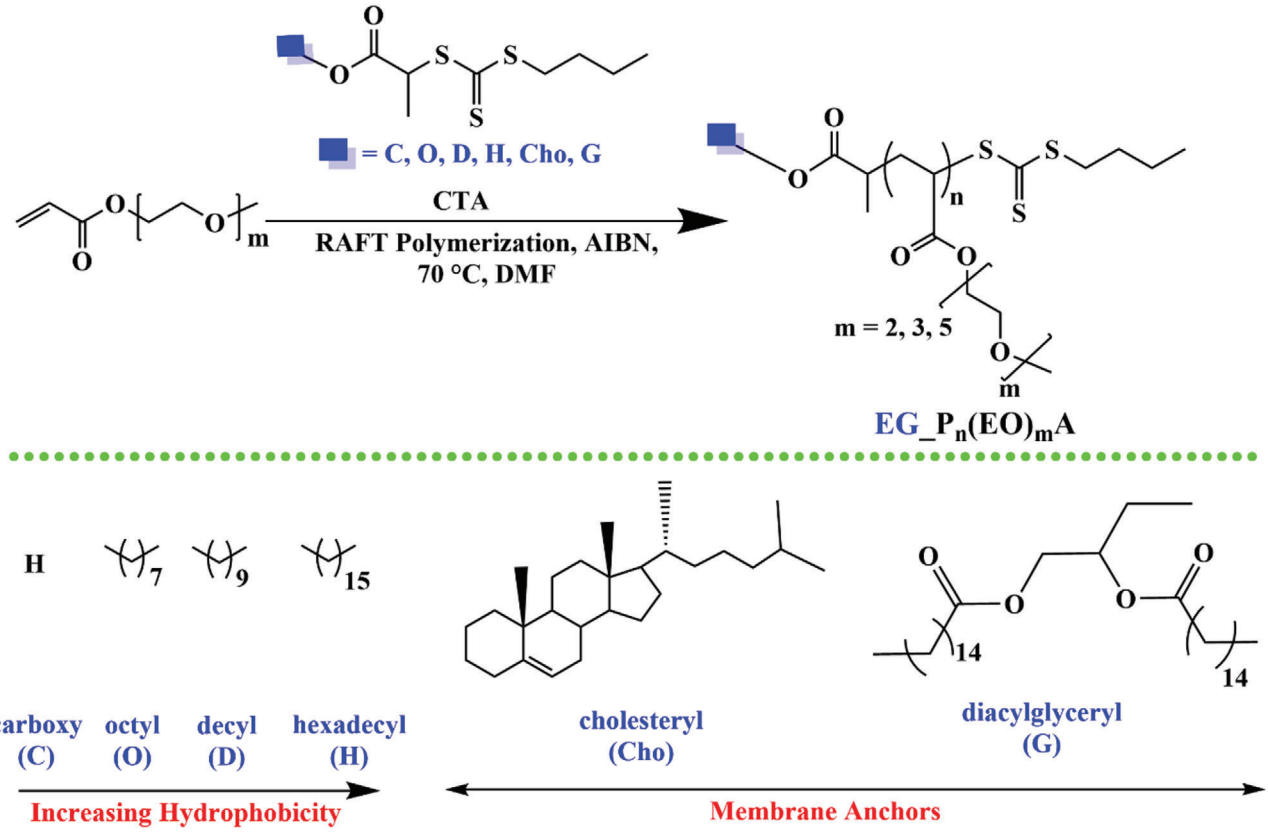

Scheme 1. Synthesis of poly(oligo(ethylene glycol)$_m$acrylate) [EG_P$_n$(EO)$_m$A] homopolymers with different end groups (EG). The polymer name indicates the number of ethylene glycol units (m), the degree of polymerization (n) with the respective end groups C, O, D, H, Cho, and G. The chemical structure of the end groups is presented below. The chemical details with the molecular weight are shown in Table 1 and Table S1 in the Supporting Information.

We here report on the synthesis of novel lipid-polymer conjugates by reversible addition-fragmentation chain transfer (RAFT) polymerization, aiming to modulate amyloid beta (Aβ_{1-40}) fibrillation. Hydrophilic oligo-ethylene acrylates are well known for their lower critical solution temperature (LCST) behavior, tuned by their oligo-ethylene side chain length, molecular weight and end groups.[33,34] To mimic the hydrophobic nature of membrane lipids, a membrane anchor was introduced along with comparable hydrophobic alkyl chains as end groups to tune the hydrophilicity-hydrophobicity profile of the polymers and to potentially promote interactions with Aβ_{1-40} peptide during the transition of polymer. We are investigating influences of the LCST transition and the polymers' end groups on Aβ_{1-40} fibrillation by modulating the amyloid beta peptide polymerization in early stages.

2. Result and Discussion

2.1. Synthesis of Polymers

The thermoresponsive poly(oligo(ethylene glycol)$_m$acrylate) [EG_P$_n$(EO)$_m$A] homopolymers with defined end groups were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization, which allows to introduce end groups (C = carboxy; O = octyl; D = decyl; H = hexadecyl; Cho = cholesteryl; G = diacylglyceryl) into well-defined polymers (**Scheme 1**, **Table 1**; Table S1, Supporting Information). The controlled polymerization was performed using the anchor containing chain transfer agents (CTA) (Scheme S1 and Figure S1–S16, Supporting Information). For the variation of hydrophilicity different ethylene glycol (EO)$_m$ units (m = 2, 3, 5) in the side chain of the monomers were used.

The polymers were fully characterized using nuclear magnetic resonance spectroscopy (^{1}H-NMR), matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF MS) and size exclusion chromatography (SEC) to ensure their end group fidelity (**Figure 1**; Figures S18–Figure S27, Supporting Information). Low polydispersities ($Đ$) ranging from 1.1 to 1.3 and molar masses ranging from 1900 to 16 700 g mol^{-1} were obtained by varying the monomer to initiator to (azobisisobutyronitrile, AIBN) - ratio ([M]/[I]) ranging from 6: 0.1 to 50: 0.1. The synthetic and experimental methods are described in detail in the Supporting Information.

2.2. Thermoresponsiveness of Polymers

The lower critical solution temperature (LCST) of the polymer was expected to influence the amyloid β peptide (Aβ_{1-40}) aggregation.[35] The hydrophilicity-hydrophobicity profile of the

Table 1. Details of the synthesized poly(oligo(ethylene glycol)$_m$acrylate) [EG_P$_n$(EO)$_m$A] polymers with the obtained characterization data.

Entry	Name	End groups (EG)	[M]/[I]	m	$M^{a}_{n,SEC}$[g mol^{-1}]	$Đ^{a)}$	$n^{b)}$	$M^{b)}_{n,\ NMR}$ [g mol^{-1}]	$T^{c)}_{cp}$ [°C]
1	C_P$_{43}$(EO)$_2$A	Carboxy (C)	50: 0.01	2	6600	1.20	43	7750	72.9
2	O_P$_{48}$(EO)$_2$A	Octyl (O)	50: 0.01	2	7600	1.27	48	8700	48.3
3	O_P$_{39}$(EO)$_3$A		50: 0.01	3	7700	1.20	39	8850	70.6
4	D_P$_{35}$(EO)$_2$A	Decyl (D)	50: 0.01	2	4650	1.16	35	6500	53.8
5	D_P$_{40}$(EO)$_3$A		50: 0.01	3	8350	1.20	40	9100	68.2
6	H_P$_8$(EO)$_2$A	Hexadecyl (H)	07: 0.01	2	2000	1.15	8	2050	> 90
7	H_P$_{39}$(EO)$_2$A		50: 0.01	2	5450	1.14	39	7250	44.4
8	H_P$_{26}$(EO)$_3$A		50: 0.01	3	6150	1.20	26	6150	58.5
9	Cho_P$_9$(EO)$_2$A	Cholesteryl (Cho)	06: 0.01	2	1600	1.19	9	2200	> 90
10	Cho_P$_{48}$(EO)$_2$A		50: 0.01	2	7250	1.20	48	9000	42.8
11	Cho_P$_{52}$(EO)$_3$A		50: 0.01	3	8450	1.30	52	11 950	57.7
12	Cho_P$_{48}$(EO)$_5$A		50: 0.01	5	7800	1.30	48	15 300	83.5
13	G_P$_{44}$(EO)$_2$A	Diacylglyceryl (G)	50: 0.01	2	6350	1.30	44	8450	39.4
14	G_P$_{11}$(EO)$_3$A		07: 0.01	3	3300	1.10	11	3200	42.6
15	G_P$_{42}$(EO)$_3$A		45: 0.01	3	7950	1.20	42	9950	52.7
16	G_P$_{52}$(EO)$_5$A		50: 0.01	5	8300	1.30	52	16 700	80.8

[a)] Molar mass (M_n) and polydispersity ($Đ$) obtained from SEC in DMF with 10×10^{-3} M $LiNTf_2$ using polystyrene (PS) as standard; [b)] Molar mass (M_n) and the degree of polymerization (n) calculated from ^{1}H-NMR spectroscopy in $CDCl_3$; [c)] T_{cp} determined in 50×10^{-3} M Na_2HPO_4, 150×10^{-3} M NaCl buffer at pH 7.4.

synthesized polymers' could be tuned via the monomers' hydrophilicity, the molecular weight, the polymer end-groups[34,36,37] and environmental factors like the presence of ions.[34] The effect of these properties on the LCST behavior of the poly(oligo(ethylene glycol)$_m$acrylate)[EG_P$_n$(EO)$_m$A] polymers was investigated by measuring the cloud point temperature (T_{cp}) by turbidimetry measurements. A Na_2HPO_4 buffer (50×10^{-3} M), 150×10^{-3} M NaCl, pH 7.4 was used to perform Aβ_{1-40} fibrillation kinetics in the presence of polymers, hence the LCST behavior of polymers was investigated under these conditions.

The influence of the polymers' end groups on the cloud point temperature (T_{cp}) was investigated for polymers containing the hydrophobic end groups (octyl = O, decyl = D, hexadecyl = H, cholesteryl = Cho, diacylglyceryl = G) compared to their hydrophilic end group (carboxy = C) containing fellow polymers. The end groups' hydrophobicity significantly decreased the T_{cp}. Hence, the carboxy group-containing polymer T_{cp} was found ≈73 °C for C_P$_{43}$(EO)$_2$A ($M_{n,NMR}$ = 7750 g mol^{-1}), and decreased to ≈48 °C for O_P$_{48}$(EO)$_2$A ($M_{n,NMR}$ = 7600 g mol^{-1}) and to ≈44 °C for H_P$_{39}$(EO)$_2$A ($M_{n,NMR}$ = 5450 g mol^{-1}) with increasing alkyl chain length of the end group. With the hydrophobic membrane anchors "Cho" and "G" as end groups a further tuning of the T_{cp} was possible. Thus, T_{cp} was reduced ≈30 and ≈34 °C for the polymers Cho_P$_{48}$(EO)$_2$A ($M_{n,NMR}$ = 7250 g mol^{-1}) and G_P$_{44}$(EO)$_2$A ($M_{n,NMR}$ = 6350 g mol^{-1}), respectively, without affecting the solubility of the polymers in the used buffer solution (**Figure 2**A, Table 1; Table S1, Supporting Information).

The hydrophilicity of the polymers' backbone produced a notable influence on the transition temperature (T_{cp}) of polymers when comparing the polymers with two to five ethylene glycol units (m = 2–5) in the side chain. The hydrophilicity of the backbone restricted the collapse, therefore the transition temperature (T_{cp}) increased from 42.8 °C for Cho_P$_{48}$(EO)$_2$A to 83.5 °C for Cho_P$_{48}$(EO)$_5$A when varying m for a comparable molecular weight for the same end group "Cho" (Figure 2B). A similar behavior was also found for the "G" end group with m = 2–5, as well as other experimented polymers (O, D, H) with m = 2, 3.

The hydrophilicity of the polymers can further be tuned with the degree of polymerization (n). Low molecular weight polymers ($n \approx 10$) showed no collapse up to 90 °C for the O, D, H, Cho, G end groups, except for the G_P$_{11}$(EO)$_3$A polymer (T_{cp} = 42.8 °C). The investigated factors (end groups, m, n) of poly(oligo (ethylene glycol)$_m$acrylate)[EG_P$_n$(EO)$_m$A] polymers on the polymers' transition behavior was consistent with literature.[33] In some cases, especially for those polymers bearing short (EO)$_m$-side chains and the Cho-end groups (Figure 2B) comparably broad transitions were observed. In line with literature, pre-transition of already coiled polymers before the T_{cp} are assumed.[38] Additional information regarding all experimental polymers' T_{cp} is mentioned in Table 1 and Table S1 in the Supporting Information.

2.3. Thermoresponsive Polymers and Aβ_{1-40} Fibrillation

As the polymers' transition-tepmeratures (T_{cp}) are tunable by the number of ethylene glycol units (m), their molecular weight (n) and end groups, and consequently the influence of EG_P$_n$(EO)$_m$A polymers on Aβ_{1-40} fibrillation were investigated, with a focus on the impact of the "Cho" and "G" end groups. The fibrillation kinetic assays of Aβ_{1-40} peptide were performed using an equimolar (10×10^{-6} M) physical mixture of peptide and polymers at 37 °C in 50×10^{-3} M Na_2HPO_4 buffer,150×10^{-3} M NaCl, at pH 7.4, and were monitored via the Thioflavin T (ThT) fluorescence intensity with time, using standard protocols to start under experimental conditions, where mostly peptide monomers are known from standard assay conditions.[33,39]

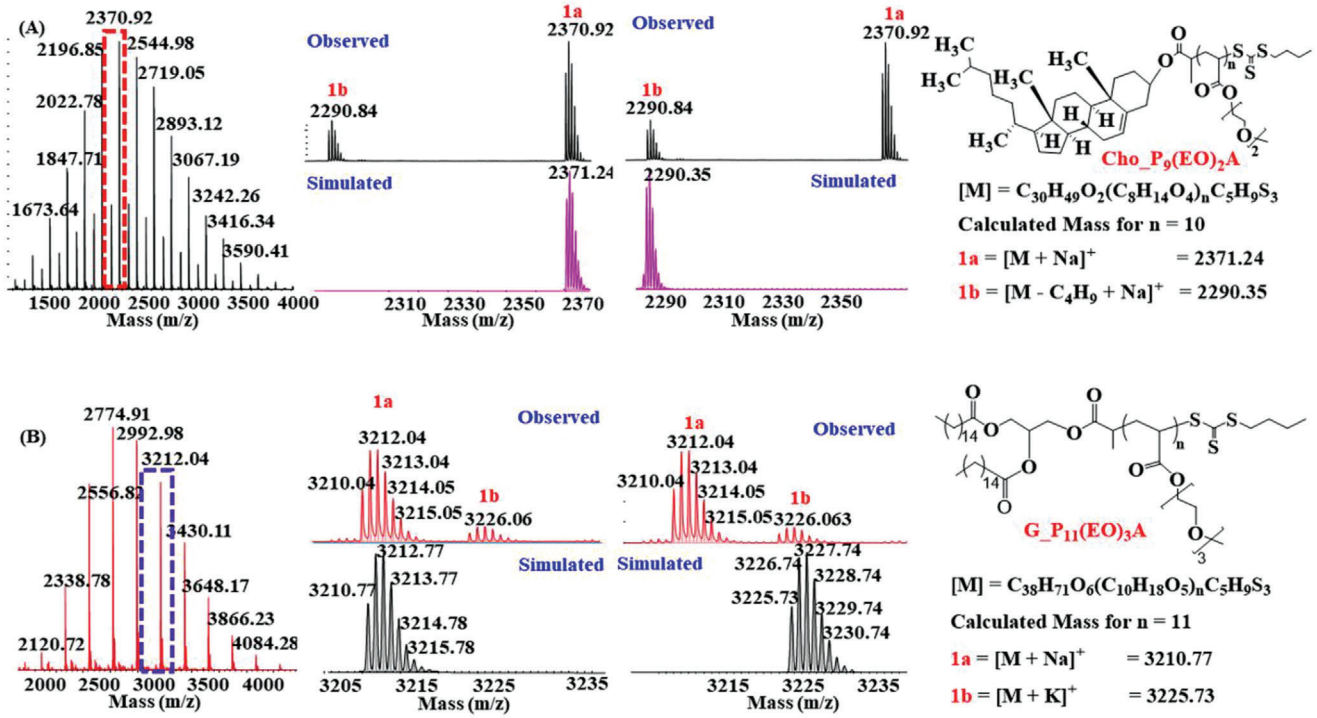

Figure 1. Full MALDI-TOF MS spectra with expansion of the assigned molecular ion peaks of the polymers. A) Cho_$P_9(EO)_2A$ and B) G_$P_{11}(EO)_3A$. The observed (upper) and simulated (lower) signals of the molecular ion peaks are presented with their chemical structure.

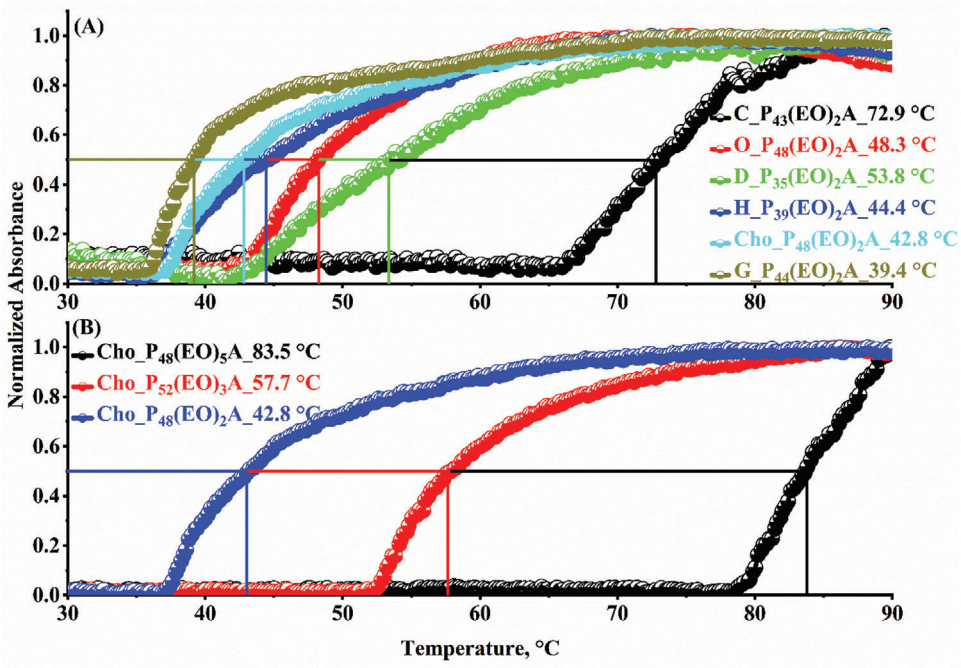

Figure 2. LCST measurements for the poly(oligo(ethylene glycol)$_m$acrylate) [EG_$P_n(EO)_mA$] polymers with carboxy (C), octyl (O), decyl (D), hexadecyl (H), cholesteryl (Cho), and diacylglyceryl (G) end groups. A) Normalized absorbance plotted against the temperature for the EG_$P_n(EO)_2A$ polymers and B) summary of the influence of the number of ethylene glycol units (m) on the T_{cp} of Cho_$P_n(EO)_mA$ polymers. Measurements were carried out in a 10×10^{-6} M solution of the polymer in Na_2HPO_4 buffer (50×10^{-3} M), 150×10^{-3} M NaCl, pH 7.4. The cloud point temperature (T_{cp}) was marked by distinctive colored lines.

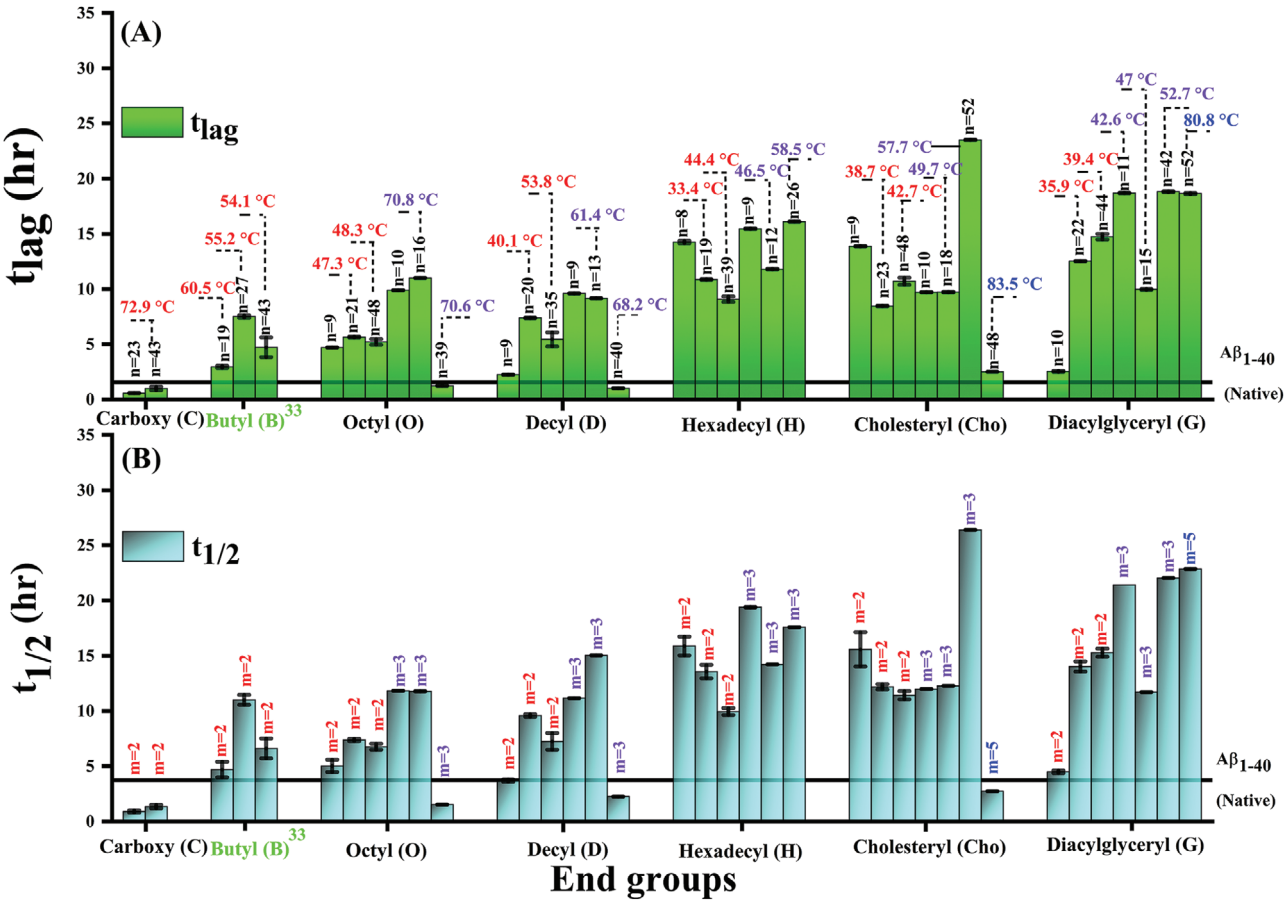

Figure 3. Influence on Aβ_{1-40} fibrillation: end groups (carboxy = C, octyl = O, decyl = D, hexadecyl = H, cholesteryl = Cho, and diacylglyceryl = G), degree of polymerization (n) and number of ethylene glycol units (m) of poly(oligo(ethylene glycol)$_m$acrylate) [EG_P$_n$(EO)$_m$A] polymers. The lag time, t_{lag} and half time, $t_{1/2}$ are obtained from a ThT monitored Aβ_{1-40} fibrillation kinetic assay in 50 × 10^{-3} M Na_2HPO_4 buffer, 150 × 10^{-3} M NaCl, pH 7.4, and 37 °C in the presence or absence of the polymers (horizontal black bold line). t_{lag} and $t_{1/2}$ were calculated from average values of three independent measurements with error bars indicated. The A) t_{lag} and B) $t_{1/2}$ are plotted against the respective end groups. Aβ_{1-40} fibrillation in the presence of poly(methoxy di(ethylene glycol)acrylates) bearing a butyl end group from the literature were compared.[33] The cloud point (T_{cp}), ^{1}H-NMR determined A) "n" and B) "m" of the corresponding polymers are mentioned on the top of the bar with different colors. The undefined T_{cp} indicates a T_{cp} above 90 °C.

To investigate the hydrophobic membrane anchors the influence on Aβ_{1-40} fibrillation the "Cho" and "G" end group-containing polymers were compared to the other hydrophobic end groups (O, D, H) containing polymers, also including their hydrophilic (C) counterpart. The fitted ThT time evolution provided the lag time (t_{lag}) and half time ($t_{1/2}$) (time to reach 50% completion of aggregation) (see fibrillation kinetics study of Aβ_{1-40} in the Supporting Information).

On the first sight, all polymers tend to inhibit fibrillation, with those bearing the "Cho" and "G" end groups most (**Figure 3**). Primarily, a strong influence of the hydrophilicity (EO-side chain length "m") on Aβ_{1-40} fibrillation was observed. To explain the "m" effect on Aβ_{1-40} fibrillation, a set of polymers containing the diacylglyceryl (G) end group was chosen bearing two to five ethylene glycol units (m = 2–5) with comparable molecular weights (n). The half times ($t_{1/2}$) and lag times (t_{lag}) determined from the ThT fluorescence kinetics are presented in Figure 3 and Table S2 in the Supporting Information. The polymer bearing two ethylene glycol units (m = 2) (G_P$_{10}$(EO)$_2$A) showed a slight increase of t_{lag} (≈3 h) and $t_{1/2}$ (≈5 h) compared to the native Aβ_{1-40} (t_{lag} ≈ 2 h and $t_{1/2}$ ≈ 4 h), albeit being devoid of a thermoresponsive behavior within the measured temperature range. Interestingly, the polymer with m = 3 (G_P$_{11}$(EO)$_3$A) exhibited a significant increase of t_{lag} (≈19 h) and $t_{1/2}$ (≈22 h) with no significant change of the lag phase in the fluorescence kinetics, although characterized by a T_{cp} of 42.6 °C (Figure 3; Figure S28A, Supporting Information).

A pronounced retardation of fibrillation was observed for polymers containing n = 15 (G_P$_{15}$(EO)$_3$A) and n = 22 (G_P$_{22}$(EO)$_2$A), displaying a low T_{cp} of 47 and 39 °C, respectively (Figure S28B, Supporting Information). Moreover, when n was increased to approximately fifty (n ≈ 50), a substantial delay of fibrillation was observed irrespective of the hydrophilic side chains m (m = 2 and 5). Hence, a pronounce inhibitory effect was quantified as t_{lag} (≈19 h) and $t_{1/2}$ (≈23 h), although these polymers display a substantial difference between their T_{cp}s (52.7 °C for G_P$_{42}$(EO)$_3$A and 80.8 °C for G_P$_{52}$(EO)$_5$A) (Figure 3; Figure S28C, Supporting Information).

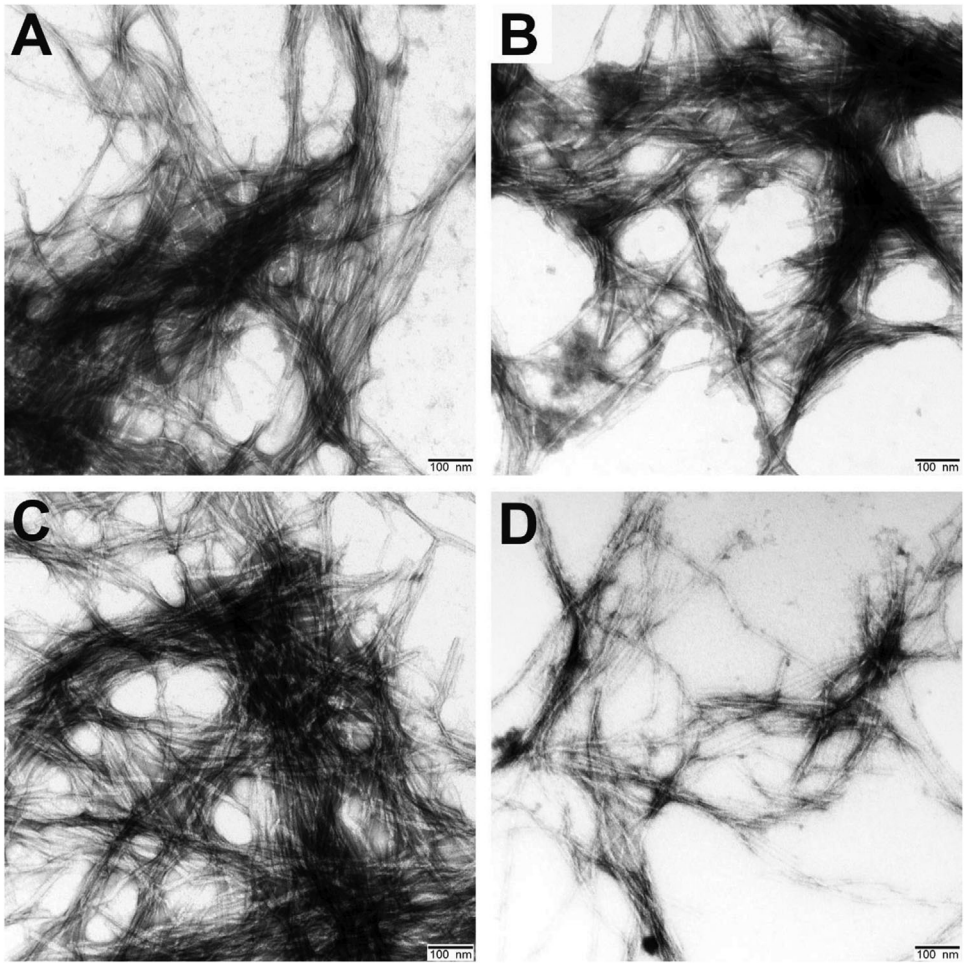

Figure 4. Aβ_{1-40} fibrillation in the presence of A) O_$P_{16}(EO)_3$A, B) H_$P_{26}(EO)_3$A, C) Cho_$P_{10}(EO)_3$A, and D) G_$P_{52}(EO)_5$A. TEM images obtained after ThT assays using uranyl acetate stain and the scale bar was 100 nm.

A very similar trend of fibrillation retardation was observed with increase of "m" for polymers bearing the cholesteryl (Cho) end groups. Upon increase of "m" from 2 to 3, a significant inhibitory behavior of fibrillation was observed for Cho_$P_{48}(EO)_2$A and Cho_$P_{52}(EO)_3$A, the molecular weights of these two polymers being comparable. Most fibrillation inhibition property among the investigated polymers was observed ($t_{lag} \approx 24$ h and $t_{1/2} \approx 27$ h) for Cho_$P_{52}(EO)_3$A, displaying a T_{cp} of 57.7 °C. On the contrary, Aβ_{1-40} fibrillation was only slightly affected by Cho_$P_{48}(EO)_5$A (T_{cp} of 83.5 °C), where fibrillation was almost unchanged by the t_{lag} (from ≈2 to ≈3 h) and $t_{1/2}$ (from ≈4 to ≈3 h). (see Figure 3; Table S2, Supporting Information).

It is notable that the polymers' inhibitory behavior showed a similar trend for octyl (O) and decyl (D) end groups with a similar degree of polymerization (till $n \approx 21$) for both $m = 2$ and $m = 3$. The inhibition of fibrillation gradually decreased with increasing molecular weight of the polymers bearing two ethylene glycol units ($m = 2$). When the degree of polymerization (n) was increased to 40, the inhibitory behavior of the polymers was reverted to an even acceleratory behavior when bearing three ethylene glycol units ($m = 3$), observing a slight acceleration of Aβ_{1-40} fibrillation ($t_{1/2}$ ≈4 h for Aβ_{1-40} to ≈1.5 h for O_$P_{39}(EO)_3$A and ≈2 h for D_$P_{40}(EO)_3$A, respectively). All results are summarized in Figure 3 and Table S2 in the Supporting Information.

Another important investigated aspect was the impact of the hydrophobic membrane anchors ("Cho" and "G") in comparison to the hydrophobic (O, D, H) end groups on Aβ_{1-40} fibrillation in the current study, where a notable increase of fibrillation inhibition was found compared to their carboxy (C) end group containing counterparts. Hence, a concomitant increase of t_{lag} and $t_{1/2}$ was observed for the O, D, H, Cho, G end groups. This pronounced inhibitory behavior is a clear indication of hydrophobic interactions with Aβ monomers or its oligomers and the end groups attached to the polymeric backbone. To probe the end groups influence on Aβ_{1-40} fibrillation, we compared a set of polymers bearing identical side chains ($m = 2$) on the backbone, with a molecular weight ranging from the 6500 to 9000 g mol^{-1}. Among this set of polymers, the strongest retardation of Aβ_{1-40} fibrillation was observed by those polymers bearing the hydrophobic membrane anchors ("Cho" and "G"), clearly exceeding those of the O, D, and H end group containing polymers. Consequently a strong retardation of fibrillation was proven by a concomitant increase of $t_{1/2}$ (≈11.50 h for Cho_$P_{48}(EO)_2$A and

15.50 h for G_$P_{44}(EO)_2$A) (Figure 3; Figure S29 and Table S2, Supporting Information).

The impact of hydrophilic-hydrophobic behavior became more eminent for the polymers possessing a longer side chain length (m = 3). Among the set of polymers, the strongest inhibition was provided by the H, Cho, and G end groups. Subsequently, the maximum increase of lag times was observed with a factor of ≈10.3 for H_$P_{26}(EO)_3$A (containing H-anchor), 15 for Cho_$P_{52}(EO)_3$A (containing Cho-anchor) and 12 for G_$P_{42}(EO)_3$A (containing G-anchor) compared to native Aβ_{1-40} in the absence of polymer (Figure 3; Table S2 in Supporting Information). The hydrophobicity provided by the O, D, H, Cho, G end groups was surely retarding Aβ_{1-40} aggregation. The trend of Aβ_{1-40} aggregation modifications in view of molecular weight (n), side chain length (m) and end groups (EG) are summarized and compared in line with the previously reported data for butyl (B) anchor containing poly(methoxy di(ethylene glycol) acrylates) (B_$P_n(EO)_m$A)[33] in Figure 3.

The polymerization of Aβ_{1-40} peptides to aggregates is a nucleation dependent process, where the hydrophobic interactions play a crucial role, induced by either homogenous or heterogeneous nucleation.[31,40,41] Such hydrophobic interactions are proposed between lipid molecules (such as cholesterol, glycerol) of the cell membrane[32,42–44] with several hydrophobic domains (Y_{10}-F_{20} and A_{30}-V_{40}) as well as the lipid moiety binding domains (E_{22}-M_{35} for cholesterol) of the Aβ_{1-40} peptide, where interactions between these domains promote the nucleation of Aβ_{1-40} fibrillation and subsequently fibril formation.[32,45]

The thermoresponsivity of polymers seems to exert only a minor effect on Aβ_{1-40} fibrillation, although being coupled to the polymer's end groups. As the inhibition of fibrillation was mainly observed during the lag and elongation phases of the Aβ_{1-40} fibril formation, we assume that the polymers modulate the nucleation behavior of Aβ_{1-40} polymerization during the aforementioned phases rather than affecting the mature fibril. The assumption is also supported by plotting the t_{lag} versus $|(T_{assay} - T_{cp})/T_{cp}|$ as presented in the supporting information (Figure S30, Supporting Information), which is displaying a similar behavior as observed in protein crystallization.[46] Further confirmation of the influence of the end groups modified EG_$P_n(EO)_m$A polymers on Aβ_{1-40} fibrillation was revealed by transmission electron microscopy (TEM) analysis of morphological details from negatively stained samples after fibrillation kinetic assays. In the presence of polymers, the aggregated samples were sharing the similar morphology of a dense, entwined long fibrils with aggregated mass and fibrils of the Aβ_{1-40} peptide, regardless of the polymer and polymer's end group (**Figure 4**; Figures S34 and S35, Supporting Information). The contribution of the polymers interactions on the aforementioned conformational transitions of monomeric Aβ_{1-40} peptide to fibrils (enriched with β-sheet structures) was probed by CD (Circular dichroism) spectroscopy, displaying a negative minimum at 218 nm.[33] Some small differences in the CD upon addition of different polymers were observed by careful analysis according to the BeStSel-algorithm[47] indicating a slightly increased amount of alpha-helicity in the mixtures with the polymers (Figure S31, Supporting Information). We believe that the hydrophobic membrane anchor (Cho and G) as well as the other hydrophobic end groups (O, D, H) of the polymers could possibly dock on such hydrophobic Aβ_{1-40} domains and postpone or delay further docking of the available monomeric Aβ_{1-40}, hence delaying the fibrillation, presumably also inducing small changes in secondary conformation during fibrillation.

3. Conclusion

We have synthesized a set of membrane anchor-containing diacylglycerol (G) and cholesterol (Cho) polymer-lipid conjugates and showed their ability to interfere with Aβ_{1-40} aggregation. Both, "Cho" and "G" containing polymers are able to strongly retard the Aβ_{1-40} aggregation, thus elongated up to a factor of ≈15 (for "Cho") and ≈12 (for "G") in comparison to native Aβ_{1-40} without polymer. We hypothesize that interactions of the hydrophobic domains of Aβ_{1-40} with "Cho" or "G" anchors on the polymers are responsible for this behavior. It is evident that the thermoresponsive nature of the polymers is affecting fibrillation to only a minor extent, although it is known that a transformation prior to the transition temperature is possible (incipient collapse).[38,48] Considering all measurements, it is clear that the hydrophobic interactions provided by the hydrophobic groups are decisive. These results motivate to further investigation of membrane-anchored polymers and their role in amyloid beta peptide fibrillation.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

N.S. synthesized and characterized monomers, RAFT agents and polymers, performed all LCST-measurements, and amyloid fibrillation kinetic assays. G.H. performed TEM assays. W.H.B. designed research and analyzed data. The paper was written by N.S. and W.H.B.

Data Availability Statement

Research data are not shared.

Keywords

amyloid beta fibrillation, lipid-polymer conjugate, stimuli-responsive polymers

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