Specific Drug Formulation Additives: Revealing the Impact of Architecture and Block Length Ratio

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Supporting Information

ABSTRACT: Combining poly(ethylene glycol) (PEG) with sequence-defined peptides in PEG–peptide conjugates offers opportunities to realize next-generation drug formulation additives for overcoming undesired pharmacological profiles of difficult small molecule drugs. The tailored peptide segments provide sequence-specific, noncovalent drug binding, and the hydrophilic PEG block renders the complexes water soluble. On the basis of a peptide sequence known to bind the photosensitizer m-tetra(hydroxyphenyl)chlorin (m-THPC) for photodynamic cancer therapy, a set of different conjugate architectures is synthesized and studied. Variations in PEG block length and amplification of the peptidic binding domain of PEG–peptide conjugates are used to fine tune critical parameters for hosting m-THPC, such as drug payload capacities, aggregation sizes, and drug release and activation kinetics.

INTRODUCTION

In recent decades, defined macromolecules and amphiphilic block copolymers have proved to be highly valuable as drug formulation additives, improving the performance of difficult drugs as well as enabling the realization of novel concepts for drug delivery and targeting.1−5 One focus of modern pharmaceutical drug design has been progressively set on hydrophobic small molecule drugs, resulting in a demand for polymer coadditives, which provide solubility, mediate bioavailability, and prevent undesired partitioning of the compounds.6−11 Despite a few very promising novel platforms,12−16 the set of investigated polymers and resulting block copolymers is largely limited to FDA approved polymers that restrict the chemical variability for block copolymer formulation additives.17,18 Bioconjugates that combine poly(ethylene glycol) (PEG) and sequence-defined peptides comprise a polymer family denoted precision polymers, which are highly information rich, cover an enormous chemical space, and have sharply definable sequence–property relationships.19−22 This class of peptide–polymer conjugates proved to have broad applicability, ranging from tailored drug solubilizers to self-assembled nano- and microstructures for cell growth scaffolds to enzymatically activable coatings or glues to anisotropic soft matter structures for molecular electronics or directed composite formation.23−36 Precision polymers exhibit rich opportunities in the field of biomedicine, enabling insights to be revealed into the behavior of multifunctional macro-molecules in complex biological environments. Recently, Wagner et al. utilized precision polymers to progress the fundamental understanding of aspects of the process that occurs in dsDNA or siRNA delivery.37 Hartmann et al. elucidated lectin binding modes of multivalent substrates to identify molecular parameters relevant for ligand binding or substrate clustering.38 Börner et al. showed that peptide–PEG conjugates could be tailored by either empirically or combinatorially assisted design to enable sequence-specific hosting of problematic drug molecules.39−41 Those specific solubilizers rendered kinase IpsiE inhibitors or m-tetra-(hydroxyphenyl)chlorin (m-THPC)42 photosensitizers with undesired pharmacological profiles readily available for biotesting or biodistribution. The platform of specific solubilizers offers opportunities to cost effectively overcome solubility issues of polar water insoluble small organic lead compounds or drug entities without tedious, cost intensive drug structure adaption cycles. However, structure–property relationships to optimize specific drug solubilizers, improving drug payload and tailoring drug/solubilizer complexation strength, are not yet well understood.

Here, we provide insights into structure–property relationships of peptide–PEG conjugates for the solubilization of an m-
Article

A detailed description of materials, instrumental, experimental procedures, and analytical data is available as Supporting Information.

■ EXPERIMENTAL SECTION

Peptide—PEG conjugates (P-PEG<sub>0.85k</sub>, P-PEG<sub>3.2k</sub>, P-PEG<sub>5.2k</sub>, P-PEG<sub>3.2k</sub>, and P-PEG<sub>5.2k</sub>) were obtained by automated solid-phase peptide synthesis on an ABI 433a peptide synthesizer (Applied Biosystems) using Tentagel-PAP resins as solid support preloaded with PEG (approximately 0.85, 3.2, and 5.2 kg/mol). After stepwise polypeptide assembly via a HBTU/NMP/piperidine protocol, conjugates were cleaved from the solid support by treatment with a mixture of 95% TFA, 4% TES, and 1% H<sub>2</sub>O, precipitated in cold diethyl ether, and dialyzed against deionized water (100–500 or 1000 Da MWCO, cellulose ester). Peptide conjugates were characterized by MALDI-ToF-MS, <sup>1</sup>H nuclear magnetic resonance spectra (<sup>1</sup>H NMR in TFA-d<sub>4</sub> and Fourier transform infrared spectroscopy (ATR-FT-IR).

For solubilization of m-THPC by peptide—PEG conjugates, m-THPC was dissolved in ethanol and mixed with the aqueous solution (1 mg/mL) and 1 mL (1.47 μmol of m-THPC) of the solution was added to solutions of each carrier (1.47 μmol) in deionized water (1 mL, pH 7). The resulting mixtures were shaken for 1 h and freeze-dried using supports preloaded with the proper PEG blocks. The inverse conjugation strategy enables the different peptide segments to be sequentially synthesized at the supported PEG blocks and the fully deprotected peptide—PEG conjugates to be liberated from the synthesis resins. The linear constructs P-PEG<sub>0.85k</sub>, P-PEG<sub>3.2k</sub>, and P-PEG<sub>5.2k</sub> were obtained in a straightforward manner. In the case of the bisvalent P2-PEG<sub>4.2k</sub>, the second peptide binding domain was linked via a flexible 6-amino hexanoic acid moiety (Fmoc-Ahx-Oh). To introduce a branching point between the three peptide domains of the tris-valent P3-PEG<sub>4.2k</sub>, a α-e-Fmoc-diprotected lysine derivative (Fmoc Lys(Fmoc)-OH) was coupled after completing the linking of the first peptide binding domain. In the ongoing SPPS, two peptide chains grow parallel with the same sequence from both the α- and ε-amino groups of the lysine residue, resulting in a Y-shaped motif. After liberation from the supports, the different peptide—PEG conjugates were isolated in a fully deprotected manner, and their chemical identities were proven by mass spectrometry (MALDI-ToF-MS) and <sup>1</sup>H NMR (Supporting Information).

To study the effect of PEG length and peptide architecture on maximum cargo capacity and payload, the set of peptide conjugates was loaded with m-THPC using an established freeze-drying methodology to force loading. For that purpose, m-THPC was dissolved in ethanol and mixed with the aqueous solutions of the corresponding bioconjugates. After freeze-drying of the mixtures and resuspension in water, the excess m-THPC could be removed by centrifugation, and drug concentration in the supernatant was determined by UV–vis spectroscopy (Figure 2).

As is evident, alterations within the peptide part of the bioconjugates seem to have more pronounced effects on m-THPC’s solubilization capacity compared to that due to changes in the length of the PEG block (Table 1). Systematically increasing the number of peptide binding domains per bioconjugate molecule, from linear monobinder to linear bis-binder to star-shaped tris-binder, resulted in a significant impact on drug solubilization efficiency. Comparing P-PEG<sub>2k</sub> with P2-PEG<sub>4.2k</sub> demonstrates that doubling the peptide segment in a linear fashion increases the molar drug payload from 1.3:4 to 1.1:4 (drug/carryer). Although P2-PEG<sub>4.2k</sub> solubilizes 0.74 mmol of m-THPC per mmol conjugate, P3-PEG<sub>4.2k</sub> reached a capacity of 0.95 mmol per
To understand the impact of amino acid sequence, hydrophobic segment has a molecular weight of 976 Da and is rather length seems to be necessary, as identical payload of 1:3.4. Interestingly, having a critical PEG payload of 1:3.3 (drug/carrier), conjugate, respectively. Whereas PEG block lengths of 3200 and 5200 g/mol, showed drug maximum cargo dramatically. Providing PEG block apparently does not in

Figure 2. UV–vis absorption spectra of m-THPC/carrIer complexes in water indicating successful drug solubilization by peptide–PEG conjugates compared to that of the free drug without formulation additives (conditions: c[conjugates] = 15 μM in water, rt, pH 7).

Table 1. Hydrodynamic Radii of Conjugate Carrier Aggregates in Water Prior to and After Loading with Maximum Amounts of m-THPC Determined by Dynamic Light Scattering and Maximum Drug Payload Capacity of Conjugate Carriers Determined by UV–vis Absorbance Spectroscopy

<table>
<thead>
<tr>
<th>carrier</th>
<th>m-THPC</th>
<th>m-THPC + P-PEG3.2k</th>
<th>payload (drug/carryer) in [mmol/mmol]</th>
<th>molar drug-to-carrier ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-PEG3.2k</td>
<td>37 ± 5</td>
<td>165 ± 22</td>
<td>0.31</td>
<td>1:3.3</td>
</tr>
<tr>
<td>P-PEG5.2k</td>
<td>13 ± 5</td>
<td>120 ± 4</td>
<td>0.30</td>
<td>1:3.4</td>
</tr>
<tr>
<td>P2-PEG5.2k</td>
<td>35 ± 3</td>
<td>110 ± 18</td>
<td>0.74</td>
<td>1:1.4</td>
</tr>
<tr>
<td>P3-PEG5.2k</td>
<td>25 ± 2</td>
<td>65 ± 7</td>
<td>0.95</td>
<td>1:1.1</td>
</tr>
</tbody>
</table>

*aConditions: DLS: c[conjugates] = 0.37 mM in water, rt, pH 7; UV–vis: c[conjugates] = 15 μM in water/EtOH 1:99, rt.*

copolymer aggregates in the absence of m-THPC within a hydrodynamic radius window of Rₐ = 13–37 nm (Table 1). Consistent with the aggregation theory of amphiphilic block copolymers, increasing the hydrophobic PEG block with the peptide segments held constant leads to smaller aggregates (cf. P-PEG₃.2k and P-PEG₅.2k). Moreover, when keeping the PEG block length unchanged, aggregate sizes become larger as the rather hydrophobic peptide segment increases due to the shift in hydrophobicity from P-PEG₃.2k to P-PEG₅.2k. P₃-PEG₅.2k shows a reduced hydrodynamic radius compared to that of P₃-PEG₅.2k which could potentially indicate a more dense packing of the hydrophobic peptides due to the compact branched topology or a change in the type of aggregate formed. As expected, the hydrodynamic radii of all solubilizer aggregates increased during loading with hydrophobic m-THPC (Table 1). The increase in the aggregate size of the loaded bioconjugates is less significant in response to increasing the molecular weight of the carriers. Whereas the smallest bioconjugate, P₂-PEG₅.2k showed the highest increase from Rₐ = 37 to 165 nm, loading of the largest solubilizer, P₃-PEG₅.2k led to the lowest increase of Rₐ from 25 to 65 nm. These results can be understood by taking into account that, in all cases, there was a rather constant ratio of peptide 7-mer binding domains per m-THPC. One drug molecule required, on average, roughly three peptide binding domains to be effectively solubilized. Because P₃- PEG₅.2k provides (with three peptide binding domains per carrier molecule) this ideal ratio, only minor changes in aggregation seem to be required. In contrast, P₂-PEG₅.2k (exhibiting a small hydrophilic PEG block and a single peptide binding domain) has to reorganize to a large extent to saturate the m-THPC molecules’ surface with an appropriate number of peptide domains and to provide sufficient PEG for colloidal stability.

Regardless of the solubilizer and the differences in drug loading and aggregation size, all m-THPC-loaded systems showed strongly reduced or no fluorescence emission (Supporting Information). This indirectly indicates the high packing density and intermolecular quenching of the m-THPC fluorophores in the aggregates. Such a silent transport mode appears to be advantageous for photodynamic therapy, as beneficial effects on shelf-life time and reduced risk during handling of the drug formulation would be anticipated. However, due to high binding affinity of m-THPC to blood plasma proteins,45,46 trans-solubilization of the sensitizer to, e.g., serum albumin takes place (Figure 3). This results in subsequent monomerization of aggregated m-THPC, reducing quenching as the drug develops into an active form.40,47 The impact of different conjugate architectures on sensitizer trans-solubilization to bovine serum albumin (BSA) as a model protein was examined by following drug activation kinetics via fluorescence emission spectroscopy. As P-PEG₅.8k failed to solubilize m-THPC, only 263 conjugates with PEG block lengths of 3200 and 5200 g/mol were compared (Figure 3). A large excess of BSA was given to aqueous solutions of conjugate carriers loaded with their respective maximum payload of m-THPC. Time-resolved fluorescence measurements show, for all solubilization systems, an increase in the fluorescence intensity, which was previously demonstrated to coincide with singlet oxygen production capability.46 Strong differences in the drug activation kinetics could be found, depending on the formulation additives used. Fluorescence emission of m-THPC solubilized by P-PEG₃.2k compared to that by P-PEG₅.2k increased considerably slower. For instance, m-THPC/P-PEG₅.2k complexes exhibited only...
Pluronic F68 as a common triblock copolymer additive to drug into the biosystem afterward should be minimized. The irradiation treatment might start 2 h after administration of desired for photodynamic therapy applications because THPC/THPC/drug load was found to have been already activated from THPC activation kinetics varied dramatically depending on the PEG3.2k obviously faster compared to activation from PEG5.2k for drug activation (Figure 3, linear or branched manner gave rise to solubilizers that The di- and trimerization of the peptide binding domains in a phobic segments, show even higher drug solubilizers proteins and drug/complexes required to promote drug trans-solubilization from aggregates to BSA. A di complexes. This could potentially cause a significant promotion of drug transfer to BSA molecules, as observed for P2-PEG5.2k and P3-PEG5.2k compared to P-PEG5.2k.

CONCLUSIONS

Tailor-made formulation additives have been studied to render m-THPC as a partially approved water-soluble photosensitizer and to potentially overcome undesired pharmacological profiles by improving bioavailability for photodynamic cancer therapy. A set of specific solubilizers was designed and synthesized based on peptide—PEG conjugates that comprise a combinatorially selected peptide segment as a binding domain to host m-THPC. The solubilizers vary systematically in PEG block length (MPEG = 850, 3200, and 5200 g/mol) and in the architecture of the peptide drug binding domain (monomeric 7-mer domain, linear dimeric domain, and branched trimeric domain). The drug formulation additives were investigated with respect to maximum payload capacities, aggregate sizes, and drug release/activation profiles. Conceptionally, the peptide segment determines drug binding and hosting. Thus, increasing the PEG block length from 3200 to 5200 g/mol did not have a significant effect on the m-THPC payload capacity. However, an adequate PEG block length seems to be required to provide sufficient water solubility and colloidal stability to the drug/solubilizer complexes, as P-PEG5.2k was not able to solubilize m-THPC. The drug payload capacity of conjugates could be doubled and tripled by amplification of the peptide segments from a linear monobinding domain to a linear bis-valent and branched trivalent domain, respectively. All solubilizers transport m-THPC in a nonactive (silent) transport form. However, drug activation occurs by trans-solubilization of m-THPC from drug/solubilizer complexes to albumins as a model of blood plasma protein. Drug activation kinetics could be precisely adjusted over a broad range according to the needs of the therapeutic approach. The activation kinetics show a pronounced dependence on the aggregate sizes of the m-THPC/conjugate complexes. It is most likely that, due to kinetic control during the forced drug loading procedure, a reduction in aggregate sizes could be achieved by increasing either the PEG block length or the valency of the peptide binding domain. A general trend was found in which rapid trans-solubilization and drug activation occurred from

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drug/solubilizer complexes as the drug payload increased and aggregate sizes decreased. This most likely reflects the relevance of the interfacial area, providing effective contacts between albumin proteins and drug/conjugate aggregates. This study underlines the opportunities for precisely adjusting peptide–PEG conjugates for solubilization of water insoluble, polar small drug molecules. It should be emphasized that combining a combinatorial approach to select peptide sequences that specifically bind small organic molecules in a noncovalent manner with different conjugate architectures can enrich the toolbox and lead to the next generation of precisely definable drug formulation additives.

■ ASSOCIATED CONTENT

* Supporting Information

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Materials, instrumentation, experimental procedures, and analytical data (PDF).

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The authors declare no competing financial interest.

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