Synthesis and properties of macrocyclic amphiphiles constructed from monodisperse oligo(ethylene glycol)

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論　文　内　容　要　旨

（NO. 1）

氏　　名	WAWRO Adam Marcin	提出年	平成 28 年
学位論文の 題　　目	Synthesis and properties of macrocyclic amphiphiles constructed from monodisperse oligo(ethylene glycol) （単分散オリゴエチレングリコールから 構築した大環状両親媒性物質の合成と物性）		

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論　文　内　容　要　旨 **(No. 2)**

Introduction

Monodisperse oligo(ethylene glycol) (mdOEG) is a unique type of PEG featuring well-defined chain length and molecular weight. This class of compounds has been increasingly popular especially in bioconjugations and chemistry of self-assembling systems, providing desired hydrophilic properties of conventional PEG without its unwanted dispersity. Unfortunately, mdOEGs are more difficult to synthesize than their common, polydisperse counterparts (e.g. PEG600), especially at multi-gram scale. Due to the limited accessibility, unique advantages of their discrete structure cannot be fully utilized in a wide spectrum of scientific applications. In this work we describe a new strategy of mdOEGs synthesis and prepare and investigate properties of various macrocyclic multi-block amphiphiles constructed from the obtained mdOEGs.

Results

1) Chromatography-free synthesis of monodisperse oligoEG tosylates

The goal of this project was to find an alternative synthetic strategy for synthesis of heterobifunctional mdOEG derivatives. We proposed and developed a chromatography-free synthesis of mdOEG monotosylates. The monotosylates are highly practical intermediates towards various bifunctional mdOEG derivatives, used for bioconjugations, synthesis of telechelic oligomers or complex functional amphiphiles. Our synthetic strategy is the first to feature full chromatography-free preparation of asymmetrically substituted mdOEG longer than tetra(ethylene glycol).

In our concept, chromatographic separations are fully replaced with liquid-liquid extractions and filtrations. Typically, symmetrically substituted PEG-type by-products, appearing inevitable during desymmetrization reactions, are removed by column chromatography. In our methodology, they are transferred to the following steps and removed at the final stage of the synthesis, when their separation can be easily accomplished.

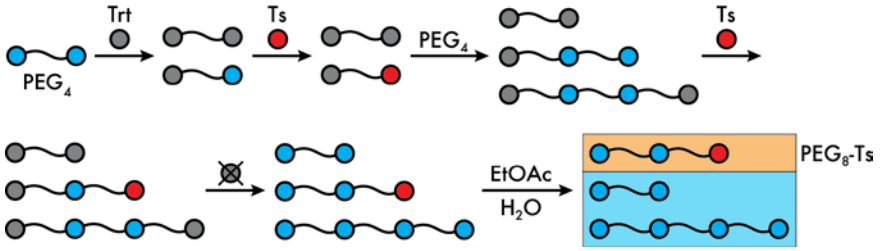

Figure 1: Idea scheme of the chromatography-free synthesis of PEG_8-Ts

Apart from the substantially simplified procedure, our method outclasses the previously reported synthetic methodologies in the fields of overall yield, cost efficiency and scalability. We prepared up to 50 g of mdOEG tosylates and assigned their purity with a quantitative RP-HPLC method. The obtained tosylates had 97–99% oligomer purity. We also discussed advantages of HPLC over mass spectrometry with respect to PEG oligomeric analysis.

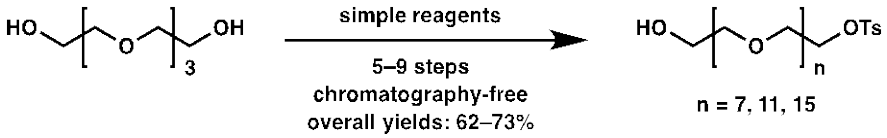

Figure 2: Summary of mdOEG tosylates synthesis.

2) Synthesis and properties amphiphilic triangle-shaped PEGs

In this project we synthesized a new class of macrocyclic multi-block monodisperse PEG amphiphiles, synthesized from the previously prepared mdOEGs. Several structural variants of PEGs were prepared and characterized, focusing of the effect of various hydrophobicity and structural topology. We investigated properties of PEGs in aqueous environment. Notably, more hydrophobic PEGs showed phase separation above certain temperature (LCST), while more hydrophilic molecules did not. The cloud point and stability of the emulsion formed from the biphasic mixture depended on the PEG topology.

In the following section we studied an effect of the presence of PEGs on the thermally induced lysozyme aggregation in buffer at 90 °C. After incubation at that temperature, lysozyme solutions lose practically all enzymatic activity, mainly due to aggregation (physical degradation) and decomposition (chemical degradation) of the protein. However, in the presence of PEG derivatives the aggregation process is significantly inhibited. All the PEGs investigated in this work exhibited strong ability to prevent the aggregation, although the efficiency depended on the exact molecular structure of the additive.

3) Covalent PEGylation with amphiphilic triangle-shaped PEGs

Monodisperse macrocyclic PEGs studied in the previous chapter were used for covalent PEGylation of a model functional peptide. In order to observe the effect of the molecular topology, two structural variants were used for PEGylation. In both cases one major isomeric product was obtained. The conjugates were purified and their structure was confirmed by site-selective digestion and mass spectrometry.

Then the effect of PEGylation on the conjugate stability was investigated. All conjugates were well soluble in water. Resistance against high temperature and extreme pH of the unmodified peptide and two PEGylated conjugates was compared. PEGylation was found to affect the conjugates stability, however the outcome was largely depending on the molecular structure of the PEG employed. This shows that the topology of amphiphiles used for PEGylation may be critical for properties of the resulting conjugate.

References:

A. M. Wawro, T. Muraoka and K. Kinbara, *Polymer Chemistry*, **2016**, 7, 2389-2394.

別　紙

論文審査の結果の要旨

ポリエチレングリコールは代表的な非イオン性水溶性ポリマーであり、低抗原性、低毒性などの特性から、特に生体関連分野での用途が拡大している。しかしながら、これまで用いられてきたポリエチレングリコールは重合によって得られる多分散性ポリマーであり、物性の精密制御を目指した分子設計の対象とはなり得なかった。このような背景から本論文は、単分散オリゴエチレングリコール誘導体の簡便合成法の開発、それを利用した新規多次元化オリゴエチレングリコール誘導体の設計、さらに、タンパク質安定化への応用について述べている。本論文は以下に示す第１章から第５章により構成されている。

第１章では、本研究を実施するに至った背景について、単分散ポリエチレングリコール合成に関する歴史的背景、ポリエチレングリコールによる生体分子修飾、ポリエチレングリコール誘導体の分子集合体形成、ポリエチレングリコールの高次元化に焦点をあて解説している。第２章では、トシル化された単分散ポリエチレングリコール誘導体をクロマトグラフィーを用いずに高効率に得る大量合成法について述べている。第３章では、前章で合成法を確立したオクタエチレングリコール誘導体を利用した、三角形状誘導体の合成とその水溶液中での会合状態を検証している。さらに、この三角形状誘導体がリゾチームに対し極めて優れた凝集抑制効果を示すことを見いだしている。第４章では、前章の三角形状誘導体および分岐型誘導体を化学結合を介してインスリンに導入し、変性に伴う会合体形成挙動を調べている。その結果、分岐型誘導体が会合体形成を抑制する効果があることを示している。第５章では、これらの研究の総括と今後の展望について述べている。

以上、本論文では、単分散ポリエチレングリコールおよびその誘導体の効率的な合成法を示すとともに，そのタンパク質安定化効果について極めて興味深い結果を示している。その成果は、生体関連機能物質開発において基礎をなす極めて重要な知見を与えるものであり、化学・生命科学の両面から今後の発展に寄与するところが大きい。すべての研究計画とその実施において主体的な役割を果たしており、自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，Wawro，Adam Marcin 提出の博士論文は，博士（理学）の学位論文として合格と認める。