Polymer 133 (2017) 205–212

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

The influence of the grafting density of glycopolymers on the lectin binding affinity of block copolymer micelles

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ARTICLE INFO

Article history:
Received 12 September 2017
Received in revised form
10 November 2017
Accepted 13 November 2017
Available online 16 November 2017

Keywords:
Self-assembly
Glycopolymers
Nanoparticles
Mixed micelles

ABSTRACT

The integration of natural carbohydrates as ligands for the targeting of specific cell types into synthetic drug delivery systems such as polymer micelles has gained increasing attention, which is based on the expression of selective receptors or lectins in the cell membrane. While several structural aspects of this interaction are well-understood, only limited knowledge exists on the impact of the grafting density of sugar groups on this interaction. In the presented approach, we created core-shell-corona micelles with comparable size and shape, but a variable density of D-mannosylated chains sticking out from the surface, and investigated their interaction with the lectin concanavalin A (Con A). The polymers were synthesized using the reversible addition fragmentation chain transfer polymerization (RAFT) providing excellent control and narrow distributions for all materials. The blocks based on butyl acrylate and *N*-acryloyl morpholine form the core and shell of the micelles, respectively, while an additional D-mannosylated block, which forms the corona, is introduced applying a previously reported post-polymerisation functionalization of a reactive bromine precursor with α-D-thiomannose. Varying the ratios of the diblock and triblock polymers in the assembly process, the density of the D-mannosylated chains could easily be adjusted in this mixed micelles approach. All micelles revealed sizes of around 50 nm (estimated by light scattering and *cryo*-TEM) and similar morphologies, an aspect that is crucial for the direct evaluation of the influence of the grafting density on the binding affinity. The subsequent binding studies with the lectin Con A revealed a clear trend that binding affinity increases with increasing sugar content and a minimum of 10% of D-mannosylated chains is required for an effective and detectible aggregation. However, it is noteworthy that the full coverage of the micelles with sugar chains is detrimental for the binding to Con A and a significant reduction of the clustering rate was observed, while a ligand density of around 50–60% was favorable for the best interaction. These finding substantiate the importance of the ligand density for the design of targeting delivery systems.

1. Introduction

Polymeric micelles (PMs) formed of amphiphilic polymers in aqueous systems gained increasing interest due to the development of modern nanoparticle formation techniques and due to their beneficial properties [1,2]. They improve the bioavailability of their loaded drugs, in particular of hydrophobic compounds, which are hardly soluble in water, and extend the drug circulation time [3]. PMs consist of an inner core, which is formed by the hydrophobic block of the amphiphilic copolymer, and of an outer shell formed by the hydrophilic part of the copolymer, which is usually used to shield the loaded drugs and to protect the micelles against recognition by the reticuloendothelial system [4]. However, PMs encounter some practical challenges, which effectively diminish their full potential. The uptake of the micelles is for example hampered by the surface shielding (e.g. by polyethylene glycol), which is implicitly necessary to prevent undesired immunoreactions and to ensure enhanced circulation time of the PMs [5].

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https://doi.org/10.1016/j.polymer.2017.11.028
0032-3861/

However, to improve the efficiency and the specificity of drug delivery into desired cells, some major efforts were undertaken based on the use of PMs. For instance, internal or external triggers can be used to enhance the pharmacological properties of PMs [6,7]. The demanded release of loaded drugs in micelles was successfully shown by the use of internal differences in the pH value and the redox environment between healthy cells and pathological structures [8] as well as by external ascendancies, such as light [9], ultrasound [10] or temperature [11]. However, when it comes to targeting of pathological structures *in vivo*, the passive approach lacks efficiency due to the random nature of this approach. Active targeting includes molecules that interact with receptors, which are either unparalleled or overexpressed on the surface of the targeted cells [2]. Progress in specific targeting finally resulted in first preclinical tests of PMs with targeting moieties towards $\alpha_v\beta_3$ integrin [12], the epidermal growth factor (EGF) [13] or the folate receptor, which are overexpressed in several cancer cells [14]. In addition, multifunctional PMs are reported that combine these targeting moieties with responsive materials acting as internal trigger [15]. Considering the large variety of potential targets, lectins have gained increasing attention due to their unique and selective interactions with sugar moieties. These binding proteins are involved in multiple biological events and can be addressed by specific carbohydrates [16], however, what is more important is that the expression of these lectins is often specific or at least very pronounced for various cell types. For instance, galectins (D-galactose binding lectins) are associated to various types of cancer [17]. Other interesting targets are for example macrophages, which exclusively express a mannose receptor or feature a high density of galactose binding lectins on their surface [18,19]. The influence of the D-mannose density on microspheres on the interaction with the lectin Con A was shown as well [20]. Reports on modified PMs demonstrate already that D-mannosylated as well as lactosylated and D-galactosylated micelles maintain a high binding affinity to their respective lectins (Con A for D-mannose and RCA-1 for lactose and D-galactose) [21–24]. Similar results were obtained for D-fructose to effectively target the GLUT5 receptor [25,26], which is responsible for the selective transport of D-fructose into cells and which is overexpressed in a number of breast cancer cells [27,28]. Several key factors, which influence the interaction between lectin and the applied glycopolymers, have been thoroughly studied and effective polymer chain lengths or ideal distances of the sugar moiety to the backbone have been evaluated [29].

Despite the wealth of information on several aspects of this interaction, the influence of the ligand density on the surface of PMs remains an almost neglected parameter for the optimization of the targeting of these drug delivery systems. Amphiphilic diblock PS-PEG copolymers with different endgroups were self-assembled in varying ratios and their affinity to murin macrophage J774 cells, which express mannose receptors involved in the internalization of *Myobacterium tuberculosis*, was investigated. For these nanocarrier system, 9% mannose-terminated PEG chains in the corona represent the maximum uptake by the receptor [30]. Here, we report a systematic study on the dependency of the surface ligand density, a D-mannosylated polymer, on the resulting biological affinity to the respective lectin Con A. The density of the sugar chains was varied between 0 and 100% by a simple but effective mixing approach, combining two block copolymers with one bearing a D-mannosylated domain in various ratios. Such an approach to create mixed micelles (MMs) has previously been used, for example, to enhance thermodynamic and kinetic stabilities, to increase the drug loading capacities, to improve the bioavailability or simply to introduce multiple functionalities [31,32]. The Stenzel group already demonstrated that such a mixing approach enables the facile variation of sugar domains in the micellar shell and tested the subsequent effect on the cell uptake [33]. However, with the change of composition a morphological transition from spherical micelles to cylindrical and other more complex structures was observed, which unfortunately limits the conclusion on the influence of the ligand density.

To overcome this issue in our attempt we first synthesized the amphiphilic diblock copolymer poly((butyl acrylate)-*block*-(*N*-acryloyl morpholine)) (P(BA-*b*-NAM)), which forms the basic spherical micelle. To introduce the mannosylated ligands this diblock copolymer was chain extended and modified to give the respective triblock terpolymer poly((butyl acrylate)-*block*-(*N*-acryloyl morpholine)-*block*-(α-D-1-*S*-mannosyl)ethyl acrylate) (P(BA-*b*-NAM-*b*-ManEA)), which features the same composition for the hydrophobic and hydrophilic domain, respectively. The use of such a triblock terpolymer to replace the initial diblock chains in the assembly should provide similar morphologies and make the D-mannose units readily available at the outside of the micelles. The resulting MMs with varying ligand densities were subsequently investigated regarding their affinities to the lectin Con A. In order to guarantee the presence of micelles, we further determined the critical micelle concentration (CMC) by dynamic light scattering (DLS) and spectroscopic measurements using Nile Red as an indicator.

2. Results and discussion

2.1. Synthesis of the polymers

Reversible deactivation radical polymerizations (RDRP), such as the RAFT process, opened a versatile route towards functional and complex architectures [34–36]. Despite this continuous effort, the direct synthesis of amphiphilic block copolymers including glycosylated domains remains a challenge due to the limited solubility of the sugar groups in less polar solvents and only a few examples are found in literature [37,38]. The use of protected and thus hydrophobic glycomonomers, however, involves additional deprotection steps, which can be rather harsh to guarantee a complete removal and cause undesired side effects in other domains of complex polymers. In our attempt to prepare an amphiphilic tri-block terpolymer, which comprises of several α-D-mannose moieties, we followed previously reported approaches using a reactive precursor polymer bearing bromine groups (Fig. 1) [39–41].

Starting with RAFT polymerization of the hydrophobic butyl acrylate (BA) using 2-(butylthiocarbonothioylthio)propanoic acid (PABTC) as the chain transfer agent (CTA), we subsequently extended this polymer with a hydrophilic block consisting of 4-acryloylmorpholine (NAM). Both monomers conveniently provide high conversions and excellent control, which has previously been utilized to create multiblock copolymers with NAM in a one-pot procedure [42,43]. Moreover, PNAM has been shown to be biocompatible and non-toxic even at the presence of up to 12% residual monomer [44]. The rather high ratio of CTA to initiator (CTA/I = 20) and the short polymerization time (4 h, only ~ 40% of initiator consumed) guarantees a high degree of livingness (>96%, calculated from the initiator decomposition and assuming an initiator efficiency of 50%) and enables the formation of a well-defined block copolymer **P2**. The final block comprising the reactive bromine groups was introduced by the RAFT polymerization of 2-bromoethyl acrylate (BEA) using **P2** as the CTA. In the initial attempt a low ratio of CTA to initiator was used (CTA/I = 3) to compensate for the low concentration of monomer (<1 M) and to guarantee a successful polymerization. However, a good conversion of 44% was reached already after 1 h of reaction (consumption of ~13% of the initiator) and, therefore, we decided to stop the polymerization in order to maintain the good control and dispersity