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Micelle-like nanoparticles of block copolymer poly(ethylene oxide)-*block*-poly(methacrylic acid) incorporating fluorescently substituted metallacarboranes designed as HIV protease inhibitor interaction probes

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ABSTRACT

We prepared nanoparticles differing in morphology from double-hydrophilic block copolymer poly(ethylene oxide)-*block*-poly(methacrylic acid), PEO–PMA, and two types of fluorescein–[3-cobalt(III) bis(1,2-dicarbollide)] conjugates, GB176 and GB179, in alkaline buffer. GB176 molecule consists of fluorescein attached to the metallacarborane anion. In GB179 molecule, the fluorescein moiety connects two metallacarborane anions. The self-assembly is based on the unusual interaction of metallacarborane clusters with PEO blocks which form insoluble micellar cores. The GB176 containing nanoparticles are loose and irregular, while the GB179 ones are rigid and spherical. The structure of nanoparticles depends to some extent on a procedure of preparation. The micelles were studied by static and dynamic light scattering, fluorometry and atomic force microscopy. Since the metallacarborane conjugates act as potent inhibitors of HIV protease, the presented system is important from the point of view of drug delivery.

Introduction

Metallacarboranes belong to promising species in medicine [1,2]. Some years ago we started a systematic research on the physico-chemical behavior of [3-cobalt(III) bis(1,2-dicarbollide)]$^-$ anion in aqueous solutions. This was promoted by the discovery of a strong inhibition activity of several metallacarborane conjugates against HIV protease [3–5]. The inhibition activity is however strongly influenced by the complex behavior of metallacarboranes in aqueous solutions [5] and the key problem is to prepare well-defined and stable dispersions of the boron containing compounds in water. From this point of view, the detailed knowledge of the metallacarborane self-assembly is a challenging task [6–10]. Our research covers studies of parent metallacarboranes [11] and (metalla)carborane conjugates [12–14] in water to their interaction with surfactants [13], cyclodextrins [14,15], phospholipid membranes [14] and with high-molar-mass hydrophilic polymers [16]. It was recently published that [3-cobalt(III) bis(1,2-dicarbollide)]$^-$ interacts with high-molar-mass poly(ethylene oxide), PEO, in aqueous solutions forming an insoluble but stimuli-responsive complex [16]. This is of importance because PEO is frequently used in medical applications [17–19].

In this paper, we investigate how to make use of this interaction for increasing the solubility of metallacarboranes in aqueous systems. We are focused on a physico-chemical behavior of boron cluster containing systems in water and their biological evaluation should be a task for a separate research. We used two types of fluorescein–[3-cobalt(III) bis(1,2-dicarbollide)] conjugates, GB176 (one metallacarborane cluster bound to fluorescein molecule) and GB179 (two metallacarborane clusters bound to fluorescein molecule; structures shown in Fig. 1), as model systems. The conjugates exhibit the HIV protease inhibition activity [20], their solubility in aqueous solutions is restricted and they form large aggregates in water, stability of which depends on pH due to the presence of fluorescein part. Details on their synthesis and behavior in aqueous solutions are described in Ref. [14].

Here we should point out several features of the probes, which are important when we want to put our study in a broader perspective. The probes represent single (GB176) and double (GB179) cluster compounds designed as HIV protease inhibitors [3–5], where the latter seem to be more promising in view of pharmacological applications [3–5,20]. Recent data suggest that the chemical structure of the "nonboron" linker, such as bulkiness, charge, and hydrophobicity can significantly influence both inhibition activity

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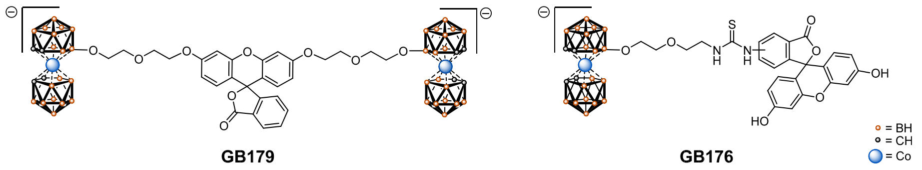

Fig. 1. Chemical structure of GB176 and GB179.

and mechanism [3–5]. As the linker, we chose the fluorescein moiety primarily due to its photoluminiscence [14]. Interestingly, its introduction to the structural patterns responsible for the inhibition of HIV protease did not deteriorate the activity substantially [20]. Besides the number of boron clusters in GB176 and GB179, we should keep in mind that also different ability to be ionized could determine the association and micellization behavior as demonstrated later. Namely, GB179 cannot lose two protons from the fluorescein part but GB176 can do in the borate buffer. This "drawback" of fluorescein will be considered in designing of fluorescent probes based on boron clusters in our future research.

We study the interaction of GB176 and GB179 with double-hydrophilic block copolymer poly(ethylene oxide)-*block*-poly(methacrylic acid), PEO–PMA, in alkaline aqueous buffers. As the buffer, we chose a dilute solution of sodium tetraborate. Despite the fact that it does not mimic biological conditions, we have the following reasons: PMA block are fully ionized and therefore soluble in the borate buffer, and metallacarboranes do not form any insoluble complexes with borate anions as they do with some amino-containing buffers such as Tris. Double-hydrophilic copolymers are usually soluble in aqueous solutions [21], but the formation of spherical micelle-like self-assemblies and their transformation in different morphologies can be provoked by the addition of ions or other agents [22–27]. The fluorescein moiety is not expected to interact with PEO–PMA copolymer, but the complexation of the parent [3-cobalt(III) bis(1,2-dicarbollide)]$^-$ cluster with PEO containing double-hydrophilic copolymers leads to the formation of nanoparticles with the core/shell structure, with PEO blocks very unusually located in the core [16].

The main goal of the study was to prepare and to investigate the nanoparticles consisting of GB176 or GB179 (inhibitors of HIV protease) and PEO–PMA based on the rather peculiar type of interaction – dihydrogen bonds [16]. Although the PMA block is not biocompatible, we choose the same copolymer as in our previous study [16] in order to check whether the presence of pendant groups of various charge attached to [3-cobalt(III) bis(1,2-dicarbollide)]$^-$ could influence the PEO/metallacarborane attraction, and to compare nanoparticle morphologies of the fluorescein conjugates with those of the parent metallacarborane. We assume that PMA blocks can be replaced without major problems by another water-soluble polymer, which does not interact with metallacarboranes. Along with the change of micelle-stabilizing blocks, we can use buffers that are closer to physiological conditions than the borate buffer. The nanoparticle formation was monitored by static and dynamic light scattering, SLS and DLS, and ^{1}H NMR spectroscopy. In addition, the time-resolved fluorescence spectroscopy was used to obtain a deeper insight in the studied systems. Last but not least, the morphology of nanoparticles was studied by AFM imaging on a mica surface.

Experimental

Materials

The GB176 and GB179 preparation and characterization have been published elsewhere [14] (structures depicted in Fig. 1). The compounds were used in form of sodium salts.

The poly(ethylene oxide)-*block*-poly(methacrylic acid), PEO–PMA, block copolymer were purchased from Polymer Source, Inc. (Dorval, Quebec, Canada). The weight averaged relative molecular weight of the PMA and the PEO block, provided by the manufacturer, are 41.0×10^3 and 30.7×10^3, respectively.

Sample preparation

The aqueous stock solutions of GB176 and GB179 were prepared by mixing of 0.8 mg of the probes with 20 mL of filtered 0.05 M solution of $Na_2B_4O_7$ (pH = 9.18) followed by half an hour of sonication and stirring overnight. As estimated by UV–Vis spectroscopy, concentrations of clear solutions of GB176 and GB179 were 5×10^{-5} M and 1×10^{-5} M, respectively. The stock solutions were not filtered and they were always sonicated before mixing with other compounds.

The GB176 and GB179 solutions containing PEO–PMA in borate buffer (pH = 9.18) were prepared as follows: (i) the GB176 and GB179 solutions were slowly titrated by PEO–PMA solution (c = 1 g/L) over a large span of PEO segment-to-probe ratios (0.5–500), (ii) 2.0 mg of solid GB176 and GB179 samples were added to 3 mL of PEO–PMA solution (c = 1 g/L) followed by stirring overnight, and (iii) 0.5, 1.0 and 8.0 mg of solid GB176 and GB179 samples were added to 1 mL of PEO–PMA solution (c = 10 g/L) in D_2O followed by stirring overnight for ^{1}H NMR experiments. As concerns samples prepared by the titration, we could not use the titration of the copolymer solution by metallacarboranes [16], because of significantly lower solubility of GB176 and GB179 comparing to sodium [3-cobalt(III) bis(1,2-dicarbollide)]. We also noticed that dialysis applied in our previous paper [16] is not a suitable method for preparation of desired nanoparticles.

Methods

Dynamic light scattering (DLS) and static light scattering (SLS)

The light scattering setup (ALV, Langen, Germany) consisted of a 633 nm He–Ne laser, an ALV CGS/8F goniometer, an ALV High QE APD detector, and an ALV 5000/EPP multibit, multitau autocorrelator. DLS data analysis was performed by fitting the measured normalized intensity autocorrelation function $g_2(t) = 1 + \beta|g_1(t)|^2$, where $g_1(t)$ is the electric field correlation function, t is the lag-time and β is a factor accounting for deviation from the ideal correlation. An inverse Laplace transform of $g_1(t)$ with the aid of a constrained regularization algorithm (CONTIN) provides the distribution of relaxation times, $\tau A(\tau)$. Effective angle- and concentration-dependent hydrodynamic radii, $R_H(q,c)$, were obtained from the mean values of relaxation times, $\tau_m(q,c)$, of individual diffusive modes using the Stokes–Einstein equation. To obtain true hydrodynamic radii, the data have to be extrapolated to a zero scattering angle. The SLS data were treated by the standard Zimm method.

Steady state and time-resolved fluorometry

Steady-state fluorescence was measured in 1 cm quartz cuvettes with a teflon stopper using a SPEX fluorolog 3-11 fluorometer. Fluorescence decays were measured by means of the time-correlated single photon counting technique on an Edinburgh Instruments ED