

Ni²⁺-NTA functionalized amphiphilic diblock-co-polymer vesicles

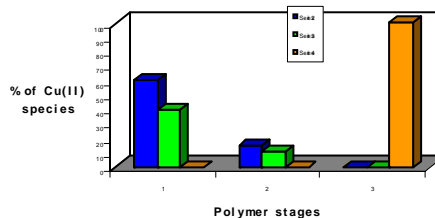
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INTRODUCTION: The amphiphilic systems which connect the worlds of synthetic polymers and natural systems are especially interesting to study the interactions between functionalized polymers and proteins in order to get new hybrid materials. The resulting molecular chimeras of polymer and protein carry the promise to interface the technical world with biological systems. Consequently, a number of experiments on these systems has already been described in the literature, such as the formation of polymer vesicles by polybutadiene-block-poly(L-glutamate)s in aqueous media.¹ In the present work we propose a new system of specifically functionalized nanovesicles for biological applications, such as crystallisation of proteins.

METHODS: Polybutadiene-block-polyethylenoxide copolymers were synthesized via anionic polymerization and subsequently quenched with molecules bearing active moieties, in an one-step procedure. The chemically active end groups offer various options for further functionalisation. The amphiphilic block copolymers were connected to N,N-Bis[(tert-butyloxycarbonyl)methyl]-L-lysine tert-Butylester (NTA.p). De-protection of the Carboxyl-groups (NTA.d) and complexation with Ni(II)/Cu(II) (NTA.d-Ni/Cu) results in a specific protein linker. The polymers were characterized by NMR, IR and SEC, while the presence of the functional groups was established by NMR and EPR. The attachment of HIS-tagged proteins to the metal-NTA-linker was studied by FCS.

RESULTS: The block copolymers (PB-PEO-NTA.d) have been complexed with Ni/Cu in order to form active metal regions, able to link His-tag proteins. In order to study if the metal attachment is directed to the NTA moiety we performed EPR measurements of the three stages in the copolymer system assembly: PB-PEO-OH, PB-PEO-NTA.p, PB-PEO-NTA.d. Both paramagnetic species are formed when the copolymer system is not functionalised with NTA (Graph 1.1), are still present when NTA is protected (Graph 1.2), but they are not anymore present when NTA is de-protected. A new paramagnetic is formed in this case (Graph 1.3).



Graph. 1: Cu(II) paramagnetic species formed by addition of Cu(OTf)₂ to: PB-PEO-OH (1), PB-PEO-NTA.p (2), and PB-PEO-NTA.d (3).

In order to link His-tag proteins to the metal coordination sphere we produced nanovesicles, by electroformation as shown in Figure 1.

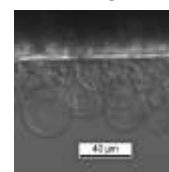


Fig. 1: Electroformation: vesicles of PB-PEO37-SA-NTA.d. Microscope: Transmission Microscope Leica DMIRE2, magnification: 20x10 Pol.1 media: aqua bidest.

The attachment of His-tag protein to the NTA-polymer linker was studied by FCS. By adding His₆-EGFP to the vesicles-Ni solution, two population were simultaneously present: one formed by the free EGFP ($\tau_D = 54 \mu s$), and another one with much bigger diffusion times (>5.4ms).

DISCUSSION & CONCLUSIONS: The diblock copolymer Polybutadiene-co-polyethyleneoxide was synthesized and functionalized in an one-step procedure. EPR is indicating that the metal is coordinated only by the NTA moiety which acts as a linking region for His-tag proteins. FCS shows that His-tag proteins attach specifically to NTA.d-Ni functionalized nanovesicles.

REFERENCES: ¹Kukula, H.; Schlaad, H.; Antonietti, M.; Förster, S. *J. Am. Chem. Soc.* **2002**, *124*, 124

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