Design of Stable Bionanoparticles by PEG based Surface Modifications

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Acetal-poly(ethylene glycol)/poly[(2-N,N-dimethylaminoethyl) methacrylate] (acetal-PEG/PAMA) block copolymer was prepared via an oxianionic polymerization technique, which was established by our laboratory. The acetal-end group was converted to an aldehyde group by an acidic hydrolysis reaction, followed by the installation of biotin moiety at the end of the PEG chain via a reductive amination reaction of biocytin hydrazide. The biotin-PEG/PAMA block copolymer thus prepared was mixed with CdSe/ZnS nano-particles (QD) in aqueous media. The obtained polymer-QD complex showed extremely high dispersion stability even in the physiological saline condition. The adsorbed biotin-PEG/PAMA block copolymer on the QD formed the tethered chain fashion on the surface to improve the dispersion stability by the steric factor of the tethered chains. Since the PEG chain end on the QD surface possessed biotin moiety, it could be utilized for not only immunohistochemistry but also various other biological applications.

Keywords: Poly(ethylene glycol) Heterotelechelic, PEG/polycation block copolymer, Oxianionic block copolymerization, CdS/ZnS quantum dot, PEG tethered chain surface, Immunohistochemistry

1. Introduction

Recently, colloidal nanoparticles, such as a gold colloid and semiconductor quantum dots (QDs), have attracted much attention due to the advantages of their size dependent optical properties. Especially, in the field of biomaterials, versatile applications have been proposed by many research groups around the world. For example, Mirkin and co-workers proposed the use of a colloidal gold system for colorimetric gene detection in 1996¹, which opened a new world for the easy sensing of single nucleotide polymorphism (SNP). Since the use of ligand-conjugated semiconductor QDs as fluorescent biolabelling reagents were reported in 1998 by the groups of Alivisatos and Nie^{2,3}, many approaches to QD applications have been done in the bioanalytical field such as DNA sequencing, tissue immuno diagnostics and single molecular imaging^{4,5,6,7}. While most nanoparticles disperse

in aqueous media based on the electrostatic repulsive force of their surface charge, the ionically-stabilized nanoparticles tend to aggregate because of the charge shielding. As a nanobiosphere, the dispersion stability under physiological conditions must be improved.

We have previously reported that PEG/polycation block copolymers stabilizes several kinds of nanoparticles such as gold⁸ and CdS QD⁹ by the coordination of the cationic segment of the nanoparticle surface to form PEG tethered chain structure on the surface. The entropically steric factor improved the stabilization of the nanoparticles even in the physiological conditions. We now report a solubilization of CdSe/ZnS core-shell type QD in aqueous media by our original biotin-PEG/poly[2-(N,N-dimethyl-amino-ethyl methacrylate) (biotin-PEG/PAMA)]¹⁰ block copolymer. The obtained biotin-PEG/PAMA modified CdSe/ZnS QD was investigated

as an immunohistochemistry.

2. Experimental Section

Biotin-PEG/PAMA block copolymer was synthesized according to our previous paper¹⁰. CdSe/ZnS QDs was kindly supplied by NOF. Biotin-PEG-b-PAMA-stabilized CdSe/ZnS aqueous dispersions were prepared as follows: Block polymers were dissolved in chloroform overnight, and QDs in chloroform solution were added. This mixture was stirred, then the solvent was removed by evaporation, and redispersed in aqueous buffer solution.

Fig. 1. Detection of type IV collagen by Biotin-PEG-b-PAMA-QDs (Rat kidney)

3. Results and Discussion

The photochemical characteristics of the biotin-PEG/PAMA-modified QDs were compared to the original QD and found that both the absorption and emission of the QDs before and after the modification reaction was almost the same, indicating the effective modification (data not shown). Using biotin-PEG/PAMA block copolymer modified QD thus prepared, immunohistochemistry was carried out with BRSAB (bridged streptavidin-biotin) technique to detect type IV collagen on Rat kidney.

Figure 1 shows a picture of the rat kidney specimen monitored by the fluorescent microscope. The type IV collagen was clearly stained by the modified QD.

Because PEG/PAMA block copolymer-modified

QDs possess PEG tethered chains on the surface, it is highly dispersion stable in aqueous media and also showed non-fouling character. Thus, it is applicable for fluorescent probes for high-performance biolabeling agent.

4. Conclusion

In summary, using PEG-b-PAMA enables stabilization of QDs in aqueous phase. Biotin-QD conjugate was suitable fluorescent probe for immunohistochemistry. By use of heterobifunctional PEG compounds, the end of the polymers can be converted into arbitrary ligands, which might be used for not only immunohistochemistry but also various other biological applications.

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