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Physicochemical characterization of the Pyridine-g-PEG copolymer at the interface

M.Fukaishi[1], T.Satomi[2], K.Ueno[1], and H.Otsuka[1, 3, *]

[1]Department of Applied Chemistry Graduate School of Science, Faculty of Chemistry, Tokyo Univ. of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo, [2]Center for Disease Biology and Integrative Medicine,Faculty of Medicine,University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, [3]National Institute for Materials Science, 1-2-1 Sengen, Tsukubashi, Ibaragi Ibaraki
Fax: +81-3-5228-8265, e-mail : h.otsuka@rs.kagu.tus.ac.jp

The novel amphiphilic graft copolymers were synthesized, which consist of methoxy-endend poly (ethylene glycol) (PEG) as a hydrophiphilic segment and pyridine as a hydrophobic and metal affinity segment (Py-*g*-PEG). Py-*g*-PEGs formed micelles and their physicochemical properties were intensively investigated in terms of critical micelle concentration, critical surface tension and micelle size. Furthermore, the gold nanoparticles (Au-NPs) were prepared using the Py-*g*-PEG polymeric micelles. Py-*g*-PEG has attained the long term interfacial stability by employing the multi-point adsorption of the pyridine unit on the gold surface. Usually, Au-NPs are prepared by thiol derivative, which are well-known to readily lose the dispersion stability under oxidative condition. Note that Au-NPs protected by Py-*g*-PEG were drastically enhanced their dispersion stability under air oxidative and high ionic strength conditions.

Key words: amphiphilic graft copolymers, polymeric micelles, gold nanoparticles.

1. INTRODUCTION

In these days, diagnostics have attained great progress particularly in detection sensitivity. Metal and semiconductor nanoparticles have been investigated to use in biomedical diagnostics, because these nanoparticles have quantum size effect (optical characteristics, high specific surface area etc[1]). But those bioconjugate nanoparticles have crucial problems such as dispersion stability in physiological condition and nonspecific adsorption of biomolecules. Those problems disturb high sensitive diagnostics. In this study, Py-g-PEG with amphiphilic character was synthesized, which enables the long-term interfacial stability by employing the multipoint adsorption of the pyridine unit[2, 3], resulting in the effective barrier to the adsorption of biomolecules at the interface. Amphiphilic graft copolymers have been interesting in behavior such as self-assembled into micelles. The micelles, unique characteristics were characterized by critical micelle concentration (cmc), surface tension, core-shell structure, and diameter. This study, further performed optimization of interfacial stability of Py-g-PEG modified surface on gold nanoparticles. Because the Py-g-PEG have the ability of multipoint anchoring through pyridine unit interfacial stability of Py-g-PEG modified surface was estimated by the dispersion stability of gold nanoparticles under physiological and oxidative conditions.

2. EXPERIMENTAL SECTION

2.1 Materials

Methacrylic acid (Wako), 4-pyridinemethanol (TCI), 4-pyridineprophanol (TCI), *N,N'*-dicyclohexylcarbodiimide (Wako), Dimethylaminopyridine (Wako), SUNBRIGHT ME-020AS (M_n=2000, a gift from NOF Corporation), 2-2'-azobis-(isobutyronitrile) (Wako), SUNBRIGHT ME-PEG-SH (M_n=2000, a gift from NOF Corporation), Pluronic F108 (BASF), $HAuCl_4 \cdot 4H_2O$ (Wako), Gold colloid solution (BB international), and hydradine monohydrate (Kanto Chemical) were used as received. The α-methoxy-ω-methacryloyl-PEG (M_n = 2080) was purified with following procedure: 50 wt. % solutions in water (Aldrich) were dried over under reduced pressure. The resulting amorphous PEG was dissolved in THF, followed drop into cold 2-propanol, and the obtained precipitate was freeze-dried from benzene, yielding white powder. All water used was purified by a Millipore unit (18mΩ resistivity).

2.2 Synthesis

Preparation of 4-Pyridylmethyl (or propyl)-methacrylate. To synthesize polypyridine-graft-PEG copolymer, 4-pyridilmethyl-methacrylate as a pyridine monomer was synthesized. Methacrylic acid (4.73 g, 55 mmol), 4-pyridine methanol (5.4 g, 50 mmol), and 4-(1-pyrrolidinyl) pyridine (740 mg, 5 mmol) were dissolved in dry dichloromethane (100 ml) in a glass vessel. After *N,N'*-dicyclohexylcarbodiimide (11.3 g, 55 mmol) was added to the solution, the reaction mixture was stirred for 1 h at room temperature. After the resulting insoluble urea was removed by filtration, the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel, hexane / ethyl acetate) yielding colorless oil (8.1 g, 46 mmol, Y. = 91.5 %). 4-pyridilpropyl-methacrylate as

a pyridine monomer was also synthesized using the same procedure as 4-pyridilmethyl-methacrylate. The yielding was colorless oil Y. = 78.2 %).

Copolymerization of Methyl-Terminated PEG macromonomers with 4-Pyridylmethyl Methacrylates. A series of Py-g-PEG were newly synthesized with radical copolymerization. As shown in scheme 1, 4-pyridylmethyl-methacrylate (410.5mg 2mmol), several amount of α-methyl-ω-methacryloyl-PEG as a macromonomer, and AIBN (1 mol% of monomer) were dissolved in dry DMF (ca.10 times amount of monomer and macromonomer mass). After the mixture was frozen and degassed 3 times, the solution was stirred for 24 h at 60°C. The reaction mixture was dropped into mixture solution (diethyl ether / 2-propanol=20/1 (10 times amount of reaction mixture)), the solution was stirred for few minutes. The resulting precipitate was separated with centrifugation (5000rpm) and freeze-dried from benzene to give white powder. Because obtained powder were polydispersity polymer (M_n/M_w= 2.0 over), the polymer was purified by repeated reprecipitation for 2 times. White powder dissolved in DMF (10 times amount of polymer mass). The solution was dropped into mixture solution (diethyl ether / 2-propanol=5/1 (10 times amount of solution)), the solution was stirred for few minutes. The resulting filtrate was separated with centrifugation (5000rpm) and freeze-dried from benzene to give white powder (Y. = 13.4%). Molecular weight distribution of yield powder were monodispersity (Mn/M_w= around 1.5).

Preparation of α-methyl-ω-methyl (4-pyridilmethyl-Carboxylate)-PEG, (single-Py-PEG). After α-methyl-ω-methyl(N-hydroxysuccinimidyl-carboxylate)-PEG (SUNBRIGHT ME-020AS) (1.14 g, 0.50 mmol), 4-pyridinemethanol (109 mg, 1.0 mmol), *N,N'*-dicyclohexylcarbodiimide (113 mg, 0.55 mmol), and 4-(1-pyrrolidinyl)pyridine (15 mg, 0.10 mmol) were added to dry dichloromethane, the mixture was stirred for 24 h at room temperature. After the resulting insoluble urea was removed with filtration, the solvent was removed under reduced pressure. The residue was precipitated from 2-propanol at 4°C, the precipitate was separated with centrifugation, freeze-dried from benzene to yield white powder (Y. = 60% 697 mg).

Analysis. ^{1}H NMR spectra were obtained using $CDCl_3$ solution with JEOL AL-300 spectrometer at 500 MHz. Tetramethylsilane was used as an internal standard. The molecular weight and molecular weight distribution of the synthesized PEG were obtained using TOSOH HLC8020 GPC equipped with a gel permeation column (TSKgel Super HZM-Z). DMF containing 10 mM triethylamine was used as an eluent.

2.3 Preparation of self-organization and characterization.

Py-g-PEG copolymer was dissolved in *N, N'*-dimethylacetamide (DMAc), a good solvent for both the hydrophilic and hydrophobic segments (5 mg / ml concentration). The solution was into a semi permeable membrane (MWCO = 12,000–14,000 g / mol) and dialyzed for 48 h against a 100-fold excess of distilled water. The dialysate was exchanged at times 2, 5, 8 and 24 h. The hydrodynamic radii and the size distribution of micelles were determined by dynamic light scattering (DLS). All measurements were carried out at 25 °C using a light scattering spectrometer (DLS-7000, Otsuka Electronics), and the scattering was carried out with a vertically polarized incident beam, at 488 nm supplied by an argon ion laser. In addition, the Py-g-PEG copolymers were analyzed by transmission electron microscopy (TEM). TEM observation was performed for the samples dried on carbon-coated copper grids. The surface tensions of aqueous solutions of the Py-g-PEG copolymers were measured with a Krüss K100 tensiometer using the Wilhelmy plate technique. Equilibrium surface tensions were taken until the change in surface tension was less than 0.01 mN m^{-1} every 1 min. The cmc and γ_{cmc} were determined from break point of the surface tension and logarithm of concentration curve. The adsorption amount of surfactants at air/liquid interface is calculated using the Gibbs adsorption isotherm equipment and other parameter was calculated.

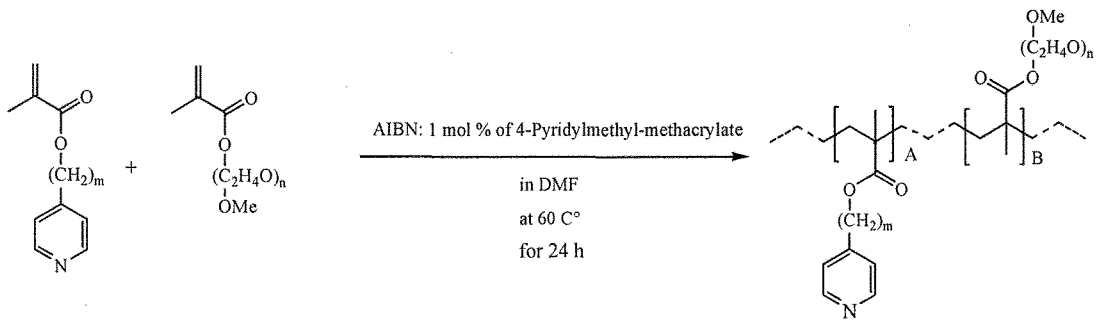
2.4 Preparation of Gold nanoparticles and characterization.

Gold nanoparticles (Au-NPs) were prepared by using, Py-g-PEG copolymer micelle. The 0.2mM of $HAuCl_4$ was added to the micelle solution with concentration of 1g/l. The solution was stirred for 1day. Hydradine monohydrate was then added to the solution. The solution was stirred for 1day. The Au NPs obtained were analyzed by TEM and UV-visible (Agilent) absorption spectroscopy. The size distribution of the Au NPs was determined from about 100 particles. The dispersion stability of Au-NPs was estimated in 1M sodium chloride aqueous solution. Absorbance change was measured by UV-vis spectra. Au-NPs protected by α-methoxy-ω-mercapto-PEG, Pluronic F108 , α-methyl-ω-methyl(4-pyridilmethyl- carboxylate)-PEG were employed to compare the stabilization.

3. RESULTS AND DISCUSSION

Pyridine-*graft*-poly (ethylene glycol) copolymers were synthesized by radical polymerization using methyl-terminated PEG macromonomer and 4-pyridylmethyl methacrylate (Scheme 1). Py-g-PEG copolymers were characterized by ^{1}H NMR. The number of each pyridine and PEG molecules per obtained graft copolymer can be controlled by initial ratio of mPEG/Py (Table 1).

According to the previously reported interfacial properties of Py-*g*-PEG (m=1)[6], suitable HLB was from 20 to 30 percent of mPEG/Py. The surface tension was



Scheme 1 Copolymerization of Methyl-Terminated PEG Macromonomers with 4-Pyridylmethyl or propyl Methacrylates.

Table 1. Parameter of synthesized Py(m)-g(mPEG/Py%)-PEG(n).

Sample name	m	n	initial ratio mPEG/Py%	mPEG/Py%	M_n	M_w/M_n	PEG unit number	Py unit number	HLB
Py(1)-g(19.7%)-PEG(43)	1	43	30	19.7	67,899	1.545	23.5	107	14
Py(3)-g(10.3%)-PEG(43)	3	43	10	10.3	75,955	1.557	18.6	181	10
Py(3)-g(23.2%)-PEG(43)	3	43	30	23.2	119,247	1.634	40.2	173	14
Py(3)-g(58.5%)-PEG(43)	3	43	60	58.5	67,498	1.511	26.9	56	16

M_n=number-average molar weight and M_w= weight-average molar weight determined by GPC
mPEG/Py was mPEG ratio to Py, which was calculated from the proton ratio of 1H NMR.
PEG and Py unit number was calculated by mPEG/Py and M_n.
HLB was calculated from glyfin method.

measured at air/liquid interface and cmc was determined. Fig. 1 shows that Py(3)-g(23.2%)-PEG(43) have the lower cmc, γ_{cmc}, A_{cmc} than Py(1)-g(19.7%)-PEG(43). Fig. 2 also shows that Py(3)-g(23.2%)-PEG(43) have the lowest value of cmc, γ_{cmc}, A_{cmc} among the sample investigated. TEM observation near cmc was performed in order to know micelle size and shape. Fig. 3 shows that micelles diameter of Py(3)-*g*(23.2%)-PEG(43) (24.6±4.6nm) was smaller than Py(1)-g(19.7%)-PEG(43) (107.9±21.4nm). As shown in Fig. 1 it is indicated that strong hydrophobic interaction for Py(3)-*g*(23.2%)-PEG(43) enhanced molecular packing within micelle[7]. As a consequence, Py(3)-g(23.2%)-PEG(43) formed monodispersed micelle. Usually, gold nanoparticles were synthesized by using adsorbed ion and self-assembling polymer. Since dispersion stability of gold nanoparticles prepared by self assembling polymer is strongly influenced by such self-assembling ability which have the close relation with micelle formation[8], Py(3)-*g*(23.2%)-PEG(43) have the suitable property among the samples investigated as protecting agent of nanoparticles.

Table 2. Parameter of adsorption and micellization.

Sample name	γ_{cmc} (mN/m)	cmc (mg/ml)	Acmc (nm2/molecules)
Py(1)-g(19.7%)-PEG(43)	44.8	0.147	1.48
Py(3)-g(10.3%)-PEG(43)	48.1	0.935	2.19
Py(3)-g(23.2%)-PEG(43)	42.7	0.115	1.27
Py(3)-g(58.5%)-PEG(43)	52.4	0.949	2.34

When gold nanoparticles were synthesized using the polymer investigated, monodispersed nanoparticles were certainly formed inside the Py(3)-*g*(23.2%)-PEG(43). This was mainly determined by the strong interaction at pyridine block in the Py-g-PEG copolymer through multiple anchoring on the gold surface. This Py-g-PEG protected Au-NPs was really stable than high ionic strength conditions (Fig.4).

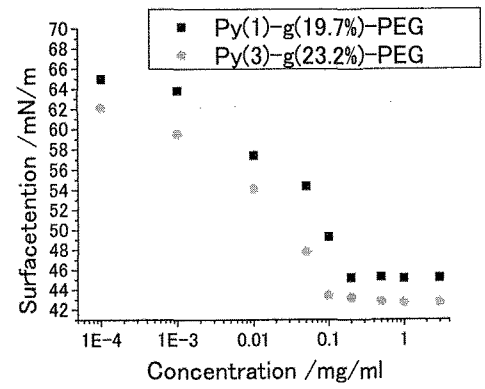


Fig. 1 Surface tension as a function of concentration for Py(1)-g(19.7%)-PEG and Py(3)-g(23.2%)-PEG.

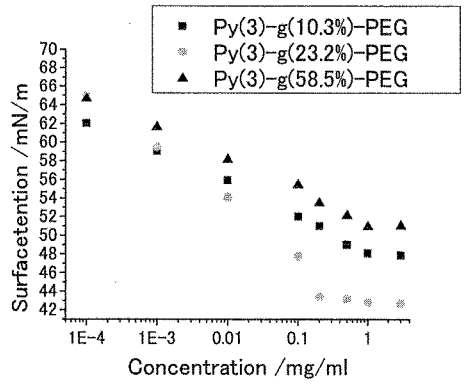


Fig. 2 Surface tension as a function of concentration for Py(3)-g(x%)-PEG.

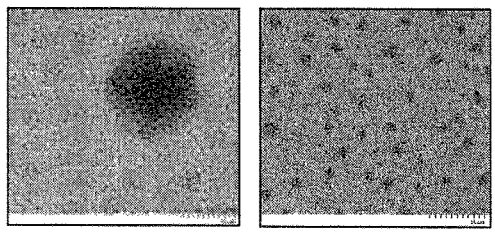


Fig .3 TEM image of Py(1)-*g*(22.1%)-PEG(43) micelles(left) and Py(3)-g(23.2%)-PEG(43).

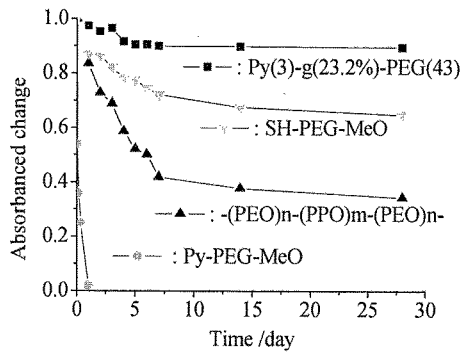


Fig. 4 Stability of Au-NPs modified by Py-g-PEG and various sorts of protecting agent in the high ionic condition (1M Sodium chloride).

Au-NPs protected by Py-g-PEG was more stable than MeO-PEG-SH and MeO-PEG-Py, suggesting the important multipoint anchoring effect for dispersion stability. Although pluronic F108 have the multipoint anchoring effect, Py-g-PEG more stabilized Au-NPs than pluronic F108. Py unit have the specific affinity interaction to gold, but pluronic F108 have only hydrophobic interaction to gold. Enhanced dispersion stability by Py-g-PEG was indicated importantly due to multiple and specific affinity of pyridine to gold surface. This observation was further interpreted under air oxidative conditions.

4. CONCLUSION

The novel amphiphilic graft copolymer (Py-g-PEG) was synthesized, and the physicochemical characterization at the interface was examined. Py(3)-*g*(23.2%)-PEG(43) have the lowest γ_{cmc} ,A_{cmc} ,cmc among the Py-*g*-PEG studied. All of series of Py-*g*-PEG (m=3) micelle size was smaller than Py(1)-g(19.7%)-PEG(43) micelles size, and the size was approximately 20nm. It is indicated that Py(3)-g(23.2%)-PEG(43) have the suitable property among the samples investigated as protecting agent of gold nanoparticles. It is possible to prepare Au-NPs by Py-g-PEG micelle. Au-NPs protected by the polymer were drastically enhanced dispersion stability under air oxidative and high ionic conditions. Py-g-PEG may have the high utility in the field of imaging, drug delivery, and high sensitive diagnosis.

5. REFERENCES

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