Synthesis and Characterization of Active Ester-Functionalized Fluorescent Polymers: New Materials for Protein Conjugation

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ABSTRACT: A novel fluorescent monomer, *N*-acryloylamido-ethyl-2-(1-pyrene)-butyrylamidophenylpropionyl amide, was synthesized and copolymerized with *N*-vinylpyrrolidone and *N*-acryloxysuccinimide to obtain a series of functional fluorescent terpolymers. Fourier transform infrared spectroscopy, ^{1}H-NMR spectroscopy, gel permeation chromatography, ultraviolet–visible spectroscopy, and fluorescence spectroscopy were used to characterize these polymers. The protein–polymer conjugation was examined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. The results indicate that conjugate formation between the terpolymers and protein resulted from the succinimidyl ester groups, which led to the formation of stable amido linkages with proteins. The results of the interactions with the COS-7 cell demonstrate that these synthesized terpolymers could attach to the surfaces of cells and emit fluorescence. These terpolymers with succinimidyl ester groups and high luminescence could be used as new materials for protein conjugation. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 777–783, 2008

Key words: biomaterials; fluorescence; proteins

INTRODUCTION

In nature, proteins have evolved for highly specialized biological functions and, as a result, are ideal for various applications in both medicine and biotechnology.[1] In the past 2 decades, protein bioconjugation has been an area of increasing interest since the pioneering work by Abuchowski et al.[2] *Bioconjugation* is the covalent binding of a protein to a synthetic or natural polymer chain to form a new macromolecule. The properties of proteins for such applications have been improved through biconjugation.[3,4] In addition, much interest has also been focused on the development of protein–polymer conjugates for use as molecular sensors for diagnostic assays, for example, to detect human immunodeficiency virus antibodies,[5,6] to study enzyme activation,[7,8] and for switches.[9,10]

In most cases, the suitable functionalization of the polymer is the first step in the conjugation. This process is the activation step, and the conjugation group is the chosen to match the available groups on proteins.[11] The conjugation groups used to date include many groups, such as meleimido,[12] aldehyde,[13] aminooxy,[14,15] and succinimidyl ester groups.[16–18] Among these groups, polymers containing succinimidyl esters are particularly appealing because they are readily reactive with amine groups (the most common side group in proteins) to form stable amido linkages with proteins under very mild conditions.

Although the chemistry for the conjugation of polymers to proteins has been widely developed, hitherto, few polymer types have been used, with poly(ethylene glycol) (PEG) remaining popular.[16] However, De Jaeghere et al.[19] demonstrated that under certain circumstances, PEG can promote the aggregation of nanoparticles after freeze drying. In this article, we propose the use of poly(*N*-vinylpyrrolidone) (PVP) as an alternative to PEG in protein–polymer biconjugation systems. PVP has excellent solubility and biocompatibility.[20,21] In addition, because of its cryo/lyoprotectant properties,[22,23] PVP might help to overcome some freeze-drying problems.

In this study, we designed and synthesized novel fluorescent copolymers containing both succinimidyl esters and fluorophore pyrene groups by free-radical copolymerization. The system had two distinct functional units. First, the succinimidyl ester groups of the copolymer served as conjugation groups for protein–polymer conjugation. Second, the fluorophore pyrene group of the copolymer provided a number of potential advantages, which included easier detection and characterization of the conjugate with fluo-

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rescence analytical techniques [fluorescence microscopy, confocal microscopy, ultraviolet–visible (UV–vis) spectroscopy, size exclusion chromatography, high-performance liquid chromatography, fluorimetry, and circular dichroism].[24] This is crucial for tracing in biological systems during biomedical assays, as the location of the material can be finely observed.

EXPERIMENTAL

Materials

N,N′-Dicyclohexyl carbodiimide (DCC), *N*-vinylpyrrolidone (NVP), and *N*-hydroxysuccinimide were all obtained from Acros Co. (Geel, Belgium). 1-Pyrenebutyl acid was purchased from Aldrich Co. L-Phenylalanine, ethylenediamine, triethylamine (TEAM), tris(hydroxymethyl)aminomethane, and thionyl chloride were purchased from Shanghai Chemical Reagents Co. (Shanghai, China). All solvents were analytical grade and were used as received. Prestained protein molecular weight marker (PPMWM) was purchased from Fermentas Co. Loading buffer (2×) was purchased from Takara Co. (Tokyo, Japan). Modified Eagle Medium and phosphate-buffered saline were purchased from Gibco Co. (USA). The China Center of Type Culture Collection (Wuhan, China) supplied the COS-7 cells.

NVP was purified by distillation under reduced pressure to remove the inhibitors before use. Azobisisobutyronitrile (AIBN) was recrystallized from methanol. Thionyl chloride, TEAM, and ethylenediamine were purified by distillation before use. Acryloyl chloride was synthesized in our laboratory. Methyl 2-amino-phenylpropionate was synthesized according to the literature.[25] Methanol, tetrahydrofuran (THF), and chloroform were dried and distilled before use according standard processes.

Synthesis of *N*-acryloxysuccinimide (NAS)

NAS was synthesized according to the method described by Pollak et al.[26] In brief, *N*-hydroxysuccinimide (11.5 g, 0.1 mol) and TEAM (11.0 g) were dissolved in 150 mL of chloroform at 0°C. To this solution, acryloyl chloride (10.0 g, 0.11 mol) was added dropwise over a 20 min period under stirring. At the end of the addition, the reaction mixture was stirred for another 20 min at 0°C. The solution was washed with ice-cold water and saturated brine, dried with $MgSO_4$, and filtered. Ethylacetate and *n*-hexane were added sequentially and slowly with stirring to the chloroform solution, which was left to stand at 0°C for several hours. The precipitated, colorless crystals were separated by filtration and washed with 10 mL of an ice-cold mixture of *n*-hexane and ethyl acetate (4 : 1), then with another 10 mL of *n*-hexane and ethyl acetate (9 : 1), and finally, with 20 mL of *n*-hexane. The crystals were dried *in vacuo* at ambient temperature to constant weight; 11.1 g (65%) was obtained at this stage (mp = 69.0–71.0°C).

Synthesis of *N*-(1-pyrene)-butyryloxysuccinimide (1)

A solution of DCC (0.43 g, 2.0 mmol) in dry THF (10 mL) was added dropwise to the stirred reaction mixture of 1-pyrenebutyl acid (0.6 g, 2.0 mmol) and *N*-hydroxysuccinimide (0.24 g, 2.0 mmol) in THF (50 mL) at 0°C. After it was stirred overnight at room temperature, the resulting mixture was filtered. The solvent was removed under reduced pressure to give compound **1** as a yellow solid, which was purified by recrystallization from ethanol.

The boldface in the following paragraphs represents the given proton's chemical shift.

Yield = 0.62 g (80.5%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.26 (m, 2H, PyCH_2**CH_2**CH_2), 2.67 (t, 2H, Py CH_2CH_2**CH_2**CO), 2.79 (s, 4H, CO**CH_2 CH_2**CO), 3.41 (t, 2H, Py**CH_2**CH_2), 7.81–8.24 (m, 9H, Py**H**). IR (KBr pellet, cm^{-1}): 3037.1 (w), 2931.7 (w), 2873.5 (w), 1812.7 (m), 1785.5 (m), 1728.4 (s), 1598.7 (w), 1374.8 (m), 1213.4 (s), 1065.5 (s), 843.7 (s), 662.9 (m).

Synthesis of 2-(1-pyrene)-butyrylamidophenylpropionate (2)

After a THF solution (50 mL) of methyl 2-amino-phenylpropionate (0.18 g, 1.0 mmol) and compound **1** (0.28 g, 1.0 mmol) was stirred at 60°C for 40 h, most of the THF was removed by evaporation. Then, a large amount of water was poured into the solution under stirring to precipitate the product. The collected product was placed *in vacuo* to give compound **2** as a pale yellow solid.

Yield = 0.33 g (73.3%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.07 (m, 2H, PyCH_2**CH_2**CH_2), 2.24 (t, 2H, Py CH_2CH_2**CH_2**CO), 2.97–3.13 (m, 2H, Ar**CH_2**CH), 3.24 (t, 2H, Py**CH_2**CH_2), 3.65 (s, 3H, O**CH_3**), 4.87 (m, 1H, ArCH_2**CH**), 5.81 (s, 1H, N**H**), 7.04–7.28 (m, 5H, Ar**H**), 7.73–8.21 (m, 9H, Py**H**). IR (KBr pellet, cm^{-1}): 3302.3 (s), 3031.2 (w), 2948.3 (w), 2864.1 (w), 1738.8 (s), 1647.2 (s), 1532.5 (s), 1427.5 (m), 1209.6 (m), 843.7 (s), 699.7 (s).

Synthesis of *N*-aminoethyl-2-(1-pyrene)-butyrylamidophenylpropionyl amide (3)

Under a nitrogen atmosphere, a solution of compound **2** (0.31 g, 0.7 mmol) in dry methanol (10 mL) was added dropwise to a stirred reaction mixture of ethylenediamine (0.6 mL, 10 mmol) and dry methanol (30 mL). After it was stirred at 40°C for 48 h, most of the methanol was removed by evaporation. Then, a large amount of water was poured into the solution under stirring to precipitate the product.

The collected product was placed *in vacuo* to give compound **3** as a pale yellow solid.

Yield = 0.25 g (74.8%).^{1}H-NMR ($CDCl_3$, δ, ppm): 2.14 (m, 2H, $PyCH_2\mathbf{CH_2}CH_2$): 2.29 (t, 2H, $PyCH_2CH_2\mathbf{CH_2}CO$), 2.61 (br, 2H, $N\mathbf{H_2}$), 3.06–3.17 (m, 2H, $Ar\mathbf{CH_2}CH$), 3.31 (t, 2H, $Py\mathbf{CH_2}CH_2$), 4.65 (m, 1H, $ArCH_2\mathbf{CH}$), 6.34 (s, 2H, 2N**H**), 7.02–7.26 (m, 5H, Ar**H**), 7.78–8.26 (m, 9H, Py**H**). IR (KBr pellet, cm^{-1}): 3285.2 (s), 3037.4 (w), 2945.4 (w), 2863.7 (w), 1638.7 (s), 1540.7 (m), 1376.8 (w), 1279.6 (w), 840.9 (s), 750.3 (w), 699.8 (m).

Synthesis of the monomer *N*-acryloylamidoethyl-2-(1-pyrene)-butyrylamidophenylpropionyl amide (PyPHA)

Under a nitrogen atmosphere, acryloyl chloride (0.28 mL, 3.5 mmol) was added slowly with a syringe to a stirred reaction mixture of compound **3** (0.24 g, 0.5 mmol) and TEAM (0.5 mL, 3.75 mmol) in THF (30 mL) at 0°C. After it was stirred overnight at room temperature, the resulting mixture was filtered to remove NEt_3·HCl. The filtrate was washed in turn with 0.1*N* HCl, brine, saturated $H_2O/NaHCO_3$, and H_2O and dried with $MgSO_4$. The solvent was removed under reduced pressure. The crude products were purified by chromatography on silica gel by elution with $CHCl_3$—CH_3OH (100:1) to give PyPHA as a pale yellow solid.

Yield = 0.19 g (71.2%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.07 (m, 2H, $PyCH_2C\mathbf{H_2}CH_2$); 2.23 (t, 2H, $PyCH_2CH_2C\mathbf{H_2}CO$); 2.95 (m, 2H, $ArC\mathbf{H_2}CH$); 3.23 (br, 6H, $PyC\mathbf{H_2}CH_2$ and $NHC\mathbf{H_2}C\mathbf{H_2}NH$); 4.54 (m, 1H, $ArCH_2C\mathbf{H}$); 5.49, 5.93, 6.13 (each, m, 1H, $C\mathbf{H_2}{=}C\mathbf{H}$); 6.07 (b, 2H, 2N**H**); 6.39 (br, 1H, N**H**); 7.02–7.24 (m, 5H, Ar**H**); 7.71–8.18 (m, 9H, Py**H**). Fast atom bombardment mass spectrometry *m*/*z* (refractive-index): 531 (11, M^+). IR (KBr pellet, cm^{-1}): 3283.8 (s), 3085.4 (w), 2943.5 (w), 1641.5 (s), 1541.7 (m), 1248.1 (w), 841.0 (s), 700.1 (m).

Synthesis of a fluorescent copolymer *N*-vinylpyrrolidone/*N*-acryloylamidoethyl-2-(1-pyrene)-butyrylamidophenylpropionyl amide copolymer (1a)]

1a was prepared by radical polymerization with AIBN as an initiator. In brief, NVP (3.6 g, 32 mmol), AIBN (0.028 g, 0.17 mmol), and PyPHA (0.039 g, 0.073 mmol) were dissolved in 12 mL of dry THF. The solution was degassed by bubbling with nitrogen for 20 min. The reaction mixture was stirred and heated to 60°C for 24 h and then cooled to room temperature. The resulting copolymer **1a** was precipitated into fivefold diethyl ether, purified by repeated reprecipitation from THF into diethyl ether three times, and dried *in vacuo* to a constant weight.

^{1}H-NMR ($CDCl_3$, δ, ppm): 1.6–3.7 (b, $C\mathbf{H_2}$, C**H**, N**H**), 7.8–8.1 (b, Py**H**). IR (KBr pellet, cm^{-1}): 3431.8 (s), 2956.6 (s), 1657.1 (s), 1435.2 (s), 1290.1 (s), 845.6 (w), 736.9 (w).

Synthesis of the fluorescent terpolymers [terpolymers of copolymers of *N*-vinylpyrrolidone (2a); *N*-acryloylamidoethyl-2-(1-pyrene)-butyrylamidophenylpropionyl amide (2b); and *N*-acryloxysuccinimide (2c)]

2a, 2b, and **2c** were synthesized in a manner similar to that followed for the synthesis of **1a** by the addition of the third monomer, NAS.

^{1}H-NMR ($CDCl_3$, δ, ppm): 1.2–4.9 (b, $C\mathbf{H_2}$, C**H**, N**H**), 7.9–8.2 (b, Py**H**). IR (KBr pellet, cm^{-1}): 3462.9 (s), 2955.2 (s), 1745.2 (s), 1657.4 (s), 1439.1 (s), 1283.4 (s), 845.2 (w), 735.4 (w).

Characterization

Steady-state fluorescence spectra were obtained on a Shimadzu RF-5301PC spectrometer (Shimadzu, Japan). The IR spectra were obtained on a Nicolet (USA) 670 Fourier transform infrared (FTIR) spectrophotometer. The mass spectra were recorded on a ZAB-HF-3F spectrometer (England). ^{1}H-NMR spectra were recorded on a Varian (USA) Mercury VX 300-MHz spectrometer. UV–vis spectra were taken on a TU-1901 spectrometer (Beijing, China) with DMF as solvent.

The molecular weights and polydispersity index values of the polymers were roughly estimated by gel permeation chromatography (GPC) analysis, with a Waters (USA) 2690-D liquid chromatograph equipped with a Shodex K803 gel column and an internal Waters 2410 refractive-index detector. Chloroform was used as eluent at a flow rate of 1.0 mL/min. A polystyrene standard with a narrow distribution was used to generate a calibration curve.

Cells were cultured in a MiTRE 4000 series culture incubator. An Axiovert 200M inverse fluorescence microscope (Zeiss Co., Germany) and an AxioCam HRO-type digital camera (Zeiss) were used to observe and record the morphology of the COS-7 cells. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS–PAGE) analyses were performed with a Bio-Rad (USA) Power PAC 300 electrophoresis system, and the gels were scanned by a Gene Genius (USA) bioimaging system.

SDS–PAGE

Protein–polymer conjugates were prepared freshly before use. Terpolymer solutions and the PPMWM solutions had concentrations of 5 and 2 mg/mL, respectively, in Tris–HCl buffers (pH = 8.0). The PPMWM solutions were then added to the copolymer solutions in different ratios (w/w), and bioconjugation was allowed for 30 min at 37°C before use.

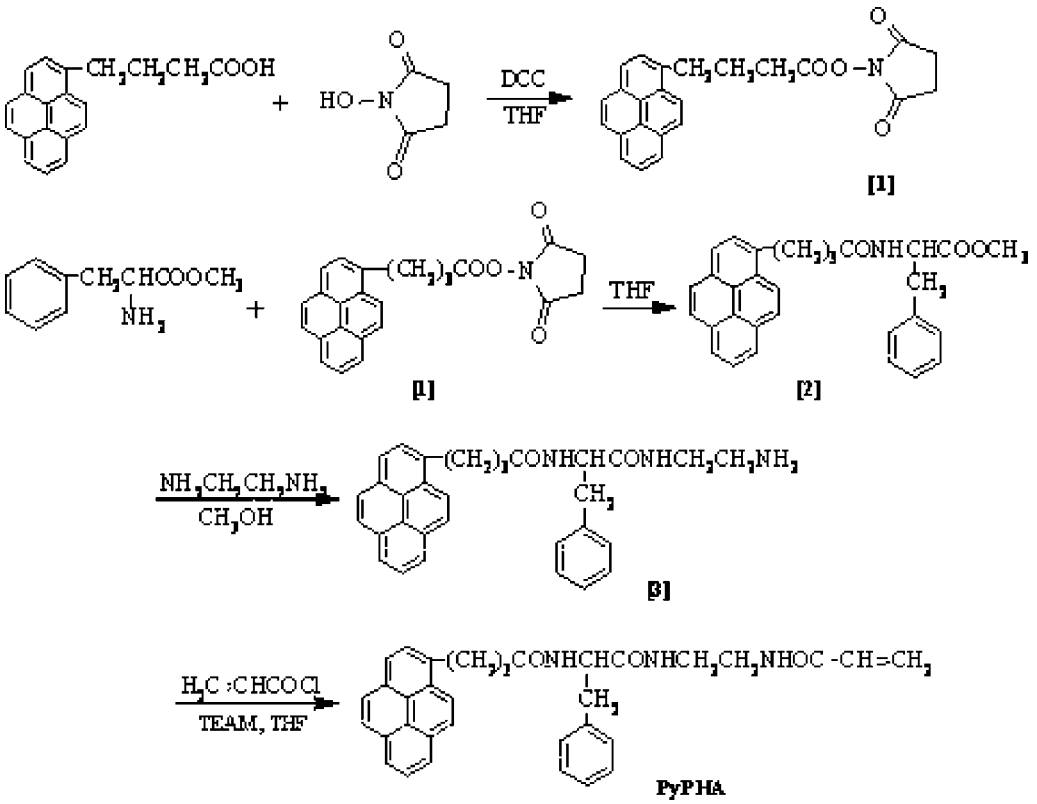

Figure 1 Scheme for the preparation of PyPHA.

To assay the ability of the terpolymer to bioconjugate PPMWM, a mixture of 5 μL of polymer/PPMWM solution and 1 μL of 2× loading buffer were loaded onto a polyacrylamide gel containing 5% stacking gel and 10% resolving gel. Electrophoresis proceeded at 180 constant volts for about 1 h. The gels were then scanned by the Gene Genius bioimaging system.

Interactions of the fluorescent terpolymers with the COS-7 cells

The balanced salt aqueous solution was composed of 140 mmol/L NaCl, 2 mmol/L $CaCl_2$, 4.2 mmol/L KCl, 0.7 mmol/L $MgCl_2$, 1 mmol/L NaH_2PO_4, and 10 mmol/L HEPES (*N*-2-hydroxyethylpiperazine-*N*-ethane-sulphonicacid), which were filtered with a 0.22-μm filter before use. First, the morphology of the COS-7 cells in the growth medium solution was taken as photos in bright field and fluorescence at an excitation wavelength of 360 nm.

Second, 100 μL of growth medium solution containing COS-7 cells were centrifugalized at conditions of 25°C and 1000 rpm/min, and the top solution was discarded. Polymer aqueous solution (20 μL, 5 mg/mL) was added to the residue of the growth medium solution, and 80 μL of the balanced salt aqueous solution was again added after 5 min, and then 5 μL of solution was taken out from the mixture solution to take photos in bright field and in fluorescence at an excitation wavelength of 360 nm.

Third, the mixture solutions were centrifugalized, and the top solution was discarded and added to 100 μL of the balanced salt aqueous solution; 5 μL of the mixture solution was taken out to take photos in bright field and in fluorescence at an excitation wavelength of 360 nm. This process was repeated.

RESULTS AND DISCUSSION

Polymer synthesis and characterization

A series of polymers, **1a, 2a, 2b,** and **2c,** were prepared by free-radical copolymerization with various comonomer molar ratios of PyPHA, NVP, and NAS in THF. The synthesis procedure of the fluorescent monomer PyPHA and polymers **1a, 2a, 2b,** and **2c** are outlined in Figures 1 and 2, respectively. These polymers were all easily dissolved in water and organic agents such as $CHCl_3$ and DMF. FTIR and ^{1}H-NMR were used to characterize these polymers. The IR spectra verified the existence of out-of-plane vibrations at 845 cm^{-1} of pyrene groups in **1a, 2a, 2b,** and **2c.** The peak at 1657 cm^{-1} was ascribed to the stretching vibration of C=O on the PVP ring and PyPHA. The succinimide carbonyl band at 1745 cm^{-1} (attributed to the succinimide C=O stretching vibration) was clearly observed in the FTIR spectra

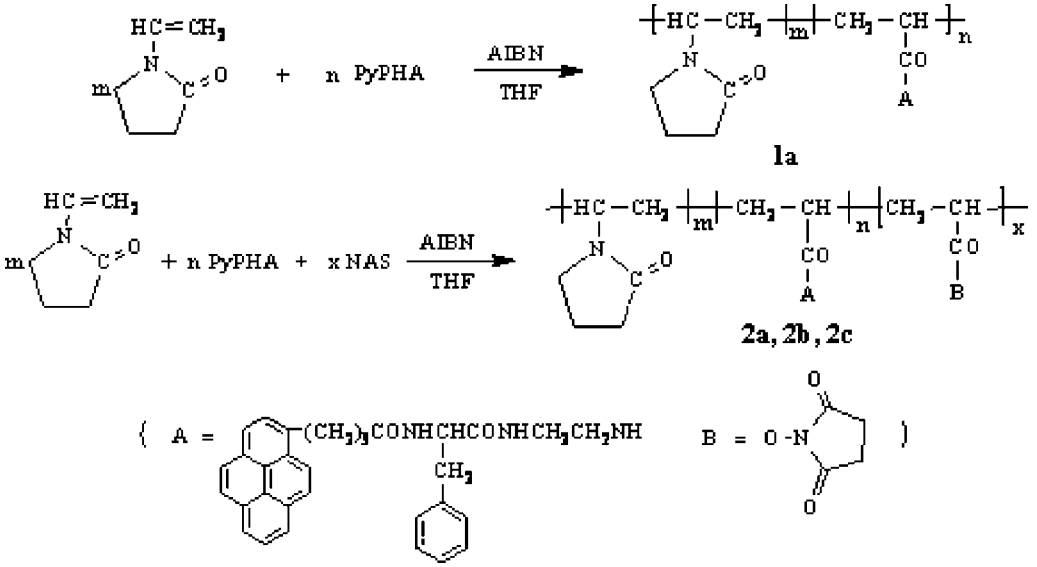

Figure 2 Scheme for the preparation of polymers **1a, 2a, 2b,** and **2c.**

of **2a, 2b,** and **2c.** This observation suggests that the terpolymers (**2a, 2b,** and **2c**) contained a significant fraction of succinimidyl ester groups.

The ^{1}H-NMR spectra of **2a, 2b,** and **2c** in $CDCl_3$ were quite similar. Signals between 1.2 and 4.9 ppm were assigned to all the protons of the CH_2, CH, and NH groups. The proton signals of the pyrene group in the polymers appeared at 7.9–8.2 ppm. The results of FTIR and ^{1}H-NMR show that PyPHA and NAS successfully copolymerized with NVP.

The exact PyPHA content of the copolymer was determined by UV–vis spectroscopy at 344 nm with a standard calibration curve experimentally obtained with 1-pyrenebutyl acid/DMF solutions (ε = 44,680 L mol^{-1} cm^{-1}; where ε is molar absorptivity or molar absorption coefficient). Table I shows the PyPHA content, weight-average molecular weight, and polydispersity index values of the synthetic polymers. The PyPHA content depended on the comonomer molar ratios and increased with increasing comonomer molar ratios added to the system. As the feed molar ratio of PyPHA to NVP increased from 1.3 : 1000 to 2.9 : 1000, the PyPHA content increased from 5.7 to 12.5 μmol/g. The molecular weights of **2a, 2b,** and **2c** as determined by GPC were 3.63 × 10^4, 4.64 × 10^4, and 4.16 × 10^4 g/mol, respectively. There was no significant difference in the polydispersity indices of **2a, 2b,** and **2c**. The data were about 2.60. The wide polydispersity index was due to the synthetic method with the free-radical polymerization.

Because of the introduction of fluorescent pyrene groups, the terpolymers (**2a, 2b,** and **2c**) showed a specific fluorescence in aqueous solutions. Figure 3 shows the fluorescent emission spectra of **2a, 2b,** and

TABLE I
Results of the Copolymerization and Characterization of the Copolymers

Polymer	PyPHA/NVP/NAS[a]	PyPHA content (μmol/g)[b]	M_w (10^4 g/mol)[c]	M_w/M_n[c]
1a	2.3 : 1000 : 0	8.9	4.09	2.70
2a	1.3 : 1000 : 15	5.7	3.63	2.63
2b	1.9 : 1000 : 15	8.8	4.64	2.35
2c	2.9 : 1000 : 15	12.5	4.16	2.77

M_w = weight-average molecular weight; M_n = number-average molecular weight.
[a] Feed molar ratio.
[b] From UV–vis absorption.
[c] From GPC analysis.

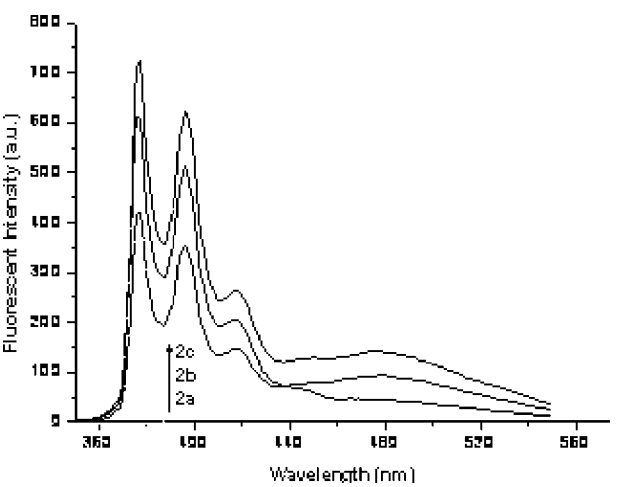

Figure 3 Fluorescent emission spectra of aqueous solutions of **2a, 2b,** and **2c** with the same concentration of 0.5 mg/mL at 25°C. The emission and excitation slit widths were all 3.0 nm. The excitation wavelength was 344 nm.

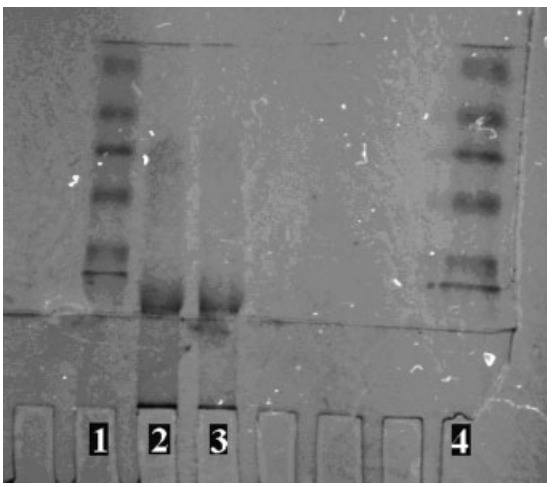

Figure 4 SDS–PAGE for the conjugation of protein with **2b** and control studies: (1) prestained protein standard PPMWM; (2,3) 2 μg of PPMWM mixed with 25 or 35 μg of **2b,** respectively; and (4) 2 μg of PPMWM mixed with 35 μg of **1a.**

2c aqueous solutions at the same concentration of 0.5 mg/mL. The emission spectra were quite similar for all of the synthesized fluorescent terpolymers. Each polymer displayed characteristic emission peaks at 377 and 396 nm and an excimer peak centered at 480 nm via direct excitation of ground-state pyrene aggregates of the polymers in aqueous solution.[27,28] The fluorescent emission intensities of **2a, 2b,** and **2c** decreased with decreasing PyPHA content, which resulted from the low concentration of pyrene groups in the copolymers at the same concentration. The advantage of using this comonomer PyPHA is that a very small percentage of it was sufficient for conferring a high fluorescence to the resulting polymers. Moreover, these properties met the requirements of fluorescence detecting and could be used to study protein–polymer conjugation.

Conjugation of the polymer to proteins

PPMWM was used as a model protein for the conjugation experiments to the functional terpolymers bearing succinimidyl ester groups in aqueous solutions. Bioconjugation was performed by the mixture of **2b** with PPMWM at weight ratios of 12.5 : 1 and 17.5:1, respectively. Analysis by SDS–PAGE (Fig. 4) clearly showed a shift to a higher molecular weight after conjugation with terpolymer **2b** (lanes 2 and 3). These results demonstrate that the polymer was covalently attached to the protein.[29]

To determine the binding sites between the polymer and protein, a control study was also performed. In this control experiment, copolymer **1a** was mixed with PPMWM at a weight ratio of 17.5:1. The SDS–PAGE bands were exactly the same as those of unmodified PPMWM (lane 4). This result indicates that there was no conjugate formation

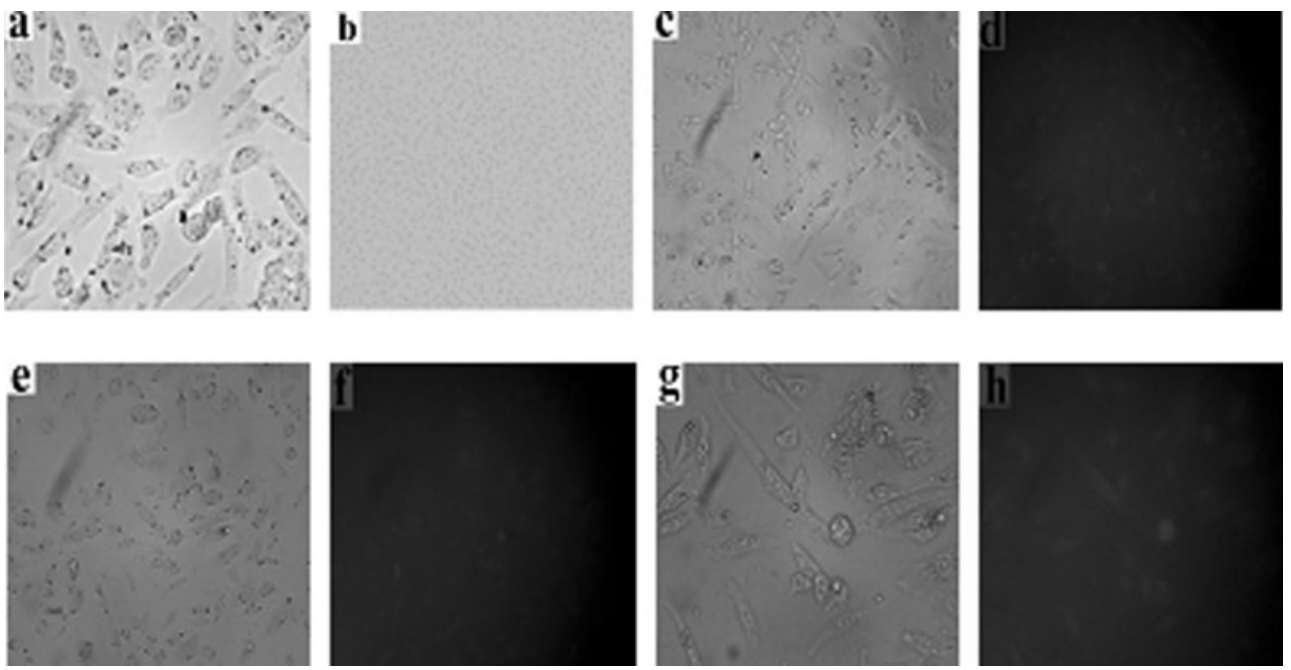

Figure 5 Photographs of COS-7 cells in the bright field and fluorescence: (a,b) COS-7 cells in the bright field and fluorescence in an aqueous solution without the polymer, (c,d) COS-7 cells in the bright field and fluorescence after the addition of 20 μL of an aqueous solution of **2a,** and (e–h) COS-7 cells in the bright field and fluorescence after they were washed and suspended in a balanced salt solution for the first and second times.

between **1a** and PPMWM. The succinimidyl ester group of **2b** was the only difference between the structures of **2b** and **1a.** Then, we concluded that conjugate formation between **2b** and protein resulted from the succinimidyl ester groups of **2b.** The succinimidyl ester groups reacted with the protein, and stable amido linkages with proteins were formed.[16]

Interactions with the COS-7 cells

To test the potential application of these functional fluorescent terpolymers as new materials for protein conjugation, we used terpolymer **2a** to test COS-7 cells. The photos of the COS-7 cell are shown in Figure 5. Figure 5(a,b) shows images of COS-7 cells in bright field and fluorescence in aqueous solution without polymer, respectively. The images are of COS-7 cells to whose growth medium solutions were added 20 μL of **2a** aqueous solution (5 mg/mL); 80 μL of balanced salt aqueous solution was again added after 5 min, and then, the solution was centrifugalized, as shown in Figure 5(c,d) in the bright field and in fluorescence, respectively. Figure 5(e–h) shows the images of COS-7 cells in the bright field and fluorescence after they were washed and suspended by a balanced salt solution for the first time [Fig. 5(e,f)] and second time [Fig. 5(g,h)]. After they were washed twice, the morphology of the COS-7 cells was still clear in the fluorescence. Considering the results of the protein–polymer conjugation, we explain this experimental result by the fact the succinimidyl ester groups of the terpolymer **2a** reacted with the surface protein of the COS-7 cells and the terpolymer **2a** covalently attached to the surface of cells.

CONCLUSIONS

The synthesis and characterization of a number of functional fluorescent terpolymers bearing succinimidyl ester groups via free-radical polymerization was described. The results of SDS–PAGE indicated that conjugate formation between the terpolymers and protein resulted from the succinimidyl ester groups, which led to the formation of stable amido linkages with proteins. The interactions with the COS-7 cells demonstrated that these synthesized terpolymers could attach to the surfaces of the cells and emit fluorescence. These terpolymers with succinimidyl ester groups and high luminescence could be used as new materials for protein conjugation.

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