Electronic Supplementary Information

Title: *Synthesis and characterization of Rhodamine B based pH-responsive fluorescent probe*

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Abstract

Rhodamine B spironolactone hydrazide (RhB-SH) was synthesized from Rhodamine B and 80% hydrazine to obtain a pH-responsive fluorescent probe, which would be used to label the polyplexes to investigate its lysosome escape ability. The structure was characterized by FTIR and ^{1}HNMR. The fluorescence of RhB-SH was investigated in different pH conditions by XRF. The results showed that RhB-SH was successfully synthesized and the fluorescence was *off* in physiological pH condition (pH=7.2-7.4) and *on* once the pH decreased to a value of lysosome condition (pH=4.5-5.0) with the excitation wavelengh of 550 nm and emission wavelength of 580 nm. The synthesized pH responsive fluorescent probe of RhB-SH was suitable to track the vector into lysosome.

Experimental

In consideration of the tumor microenvironments, stimuli responsive fluorescent probes are suggested to be helpful to track the intracellular gene delivery mechanisms [1]. RhB-SH, derived from RhB, has a spironolactone hydrazide, which results in no fluorescence. When the spironolactone hydrazide structure is opened in decreased pH, the fluorescence is recovered. The pH responsive *OFF-ON* properties have been used to track the lysosome enter and escape of gene vectors.

RhB-SH was synthesized from RhB by refluxing with 80% hydrazine at 80 °C for 2 h (Fig. 1) [2]. In detail, RhB of 0.2011 g is added into 250 mL flask with 15mL anhydrous ethanol to get a deep purple red solution. 80% hydrazine of 2 mL was added under violently stirring at room temperature to obtain a purple red solution. After refluxing for 2 h at 80 °C, the solution became light brown and yellow. The solution was evaporated to obtain light yellow powers. 1 mol/L hydrochloric acid solution of 8.3 mL was added to obtain a yellow solution. Then 1 mol/L sodium hydroxide solution was added to modulate the pH up to 9-10. After centrifuged (8000 r/min, 10 min), the precipitate was collected and washed with ultrapure water for 3 times and dried under vacuum. The obtained yellow power was characterized by

FTIR (Fig. 2), NMR (Fig. 3) and RF (Fig. 4). The new peak in Fig. 2 appeared at 1694 cm^{-1}, 2965 cm^{-1}, 2968 cm^{-1} indicated that the the formation of spiridamide structure. The new peak (Fig. 3) appeared at 4.25 ppm was assigned to amino group of spiridamide. FTIR and NMR spectra demonstrated the successfully synthesis of RhB-SH.

The fluorescence intensity of RhB-SH at 550 nm excitation increased with the decrease of pH, as shown in Fig. 4. The fluorescence of RhB-SH was very low at pH 7.0~6.38. When pH value decreased to 6.19, the fluorescence became recovery. And the fluorescence nearly completely recovered when pH decreased to 4.67. When pH further decreased to 4.32, the increase of the fluorescence was slight.

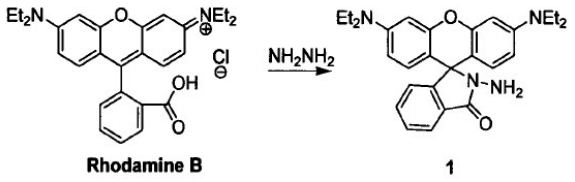

Fig. 1 Scheme of synthesis of RhB-SH

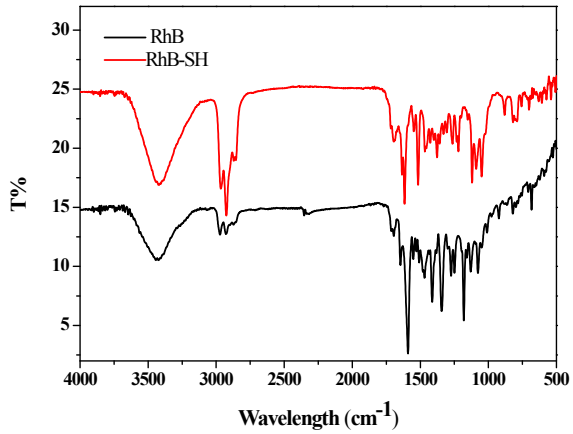

Fig. 2 FTIR spectra of RhB and RhB-SH

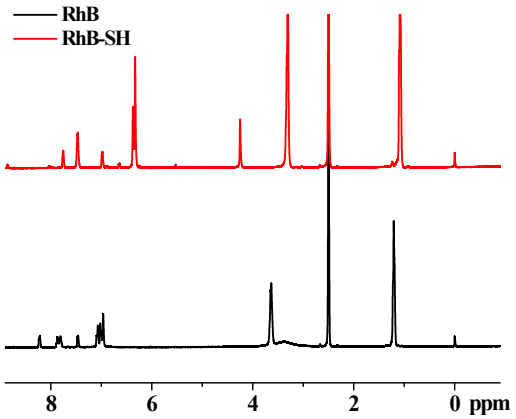

Fig. 3 [1]HNMR spectra of RhB and RhB-SH

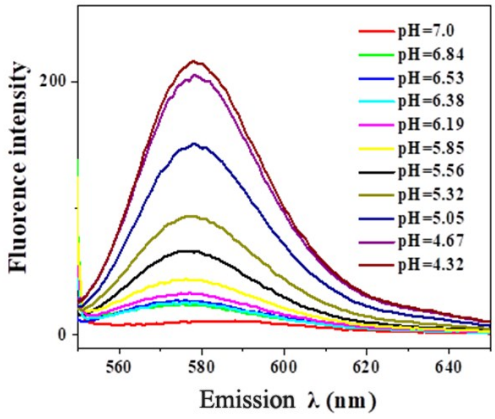

Fig. 4 Recovery of fluorescence of RhB-SH in decreased pH.

Acknowledgements

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