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A resonance light scattering ratiometry applied for binding study of organic small molecules with biopolymer

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Abstract

Resonance light scattering (RLS) technique is a creative application of light scattering signals detected by using a common spectrofluorometer, but it has drawbacks such as the fluctuation of signals caused by poorly quantified or variable factors. Herein we develop a RLS ratiometry to overcome the drawbacks of the technique and apply to measure the binding nature of organic small molecules (OSM) with biopolymer using the binding of cation porphyrins with heparin (HP) as an example. In near neutral solution, cationic porphyrins *meso*-tetrakis [(trimethylammoniumyl) phenyl] porphyrin (TAPP) and *meso*-tetra (4-methylpyridy) porphyrin (TMPyP-4) interact with heparin, resulting in hypochromatic effect, and enhanced RLS signals. Linear relationship could be established between the ratio of enhanced RLS signals at two wavelengths, where the maximum and minimum are available in the ratio curve of UV–vis spectrum of porphyrin to that of heparin–porphyrin complex, and the logarithm of heparin concentration, and thus a wide dynamic range detection method of biopolymers could be developed. In comparison with RLS method, this RLS ratiometric one is less affected by environmental conditions such as pH, ionic strength. The mechanism of these interactions was investigated based on the charge density distribution of the two porphyrin molecules and it could be concluded that the enhanced RLS intensity is proportionally promoted by the charge capacity of components in the complex. Additionally, the binding number and binding constant were measured scientifically by Scatchard plot.

Keywords: Resonance light scattering ratiometry; Organic small molecules (OSM); Biopolymers

1. Introduction

Binding study of organic small molecules (OSM) with biopolymers is very important in elucidating the nature of drug-targeted biomolecules [1,2]. In order to obtain the binding information in terms of kinetics and binding affinities, techniques such as X-ray diffraction, NMR spectroscopy, circular dichromism, ultraviolet–vis (UV–vis) molecular absorption and fluorescence spectroscopy have been traditionally available [3–5]. It has proved that Resonance Raman (RR) spectroscopy with ultraviolet excitation radiation is a valuable method [4,5], and its increased sensitivity is a good example to show that light scattering signals could have been applied sensitively to tissue studies [1], in vivo cancer diagnosis [2], immunocytology application [3], and DNA hybridization [4]. Similar to the enhanced RR spectroscopy, resonance light scattering (RLS) technique is a newly developed tool by using a common spectrofluorometer to detect enhanced light scattering signals in the assemblies of π-stacking molecules [5,6]. This technique has the advantages of simple operation and high sensitivity, showing high promise in studies of biochemistry [7–11], pharmacology [12], and molecular biology [4]. In practical application, however, the RLS signals suffer from fluctuation caused by many poorly quantified or variable factors in solution such as the incident light intensity, reagent concentration, and environmental conditions in the medium including pH, ionic strength, temperature, polarity [13]. Thus, it is necessary to improve the technique to compensate for these defaults.

Here we develop a RLS ratiometry considering that ratiometry is a good method to solve the problems posed by single-wavelength measurement because the ratiometry could provide precise data by taking the intensity ratio at two suit-

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able wavelengths [14–17]. For example, fluorescent ratiometry has been commonly utilized in sensing physiological pH, oxygen and metal ion in cells [17–21]. We find that the RLS ratiometry could differentiate the binding difference of two cation porphyrins, *meso*-tetrakis [(trimethylammoniumyl) phenyl] porphyrin (TAPP) and *meso*-tetra (4-methylpyridy) porphyrin (TMPyP-4), with biopolymer, heparin (HP), a kind of sugaramic polysaccharide, which has widely applied to prevent thrombosis of extracorporeal circuit and to mediate activation of the hemostatic system during surgery [22,23]. Based on charge density distribution of the two porphyrin molecules, we try to discuss the binding dependence on molecular structure. Such knowledge then makes it possible to do systematic structural modifications of the drug molecule to optimize the binding interaction.

2. Experimental

2.1. Materials and apparatus

Heparin (HP) solution was prepared by dissolving heparin sodium (160 IU mg^{-1}, Shanghai Chemical Reagent Plant, Shanghai, China) in water. HP working solution is 10 $\mu g\,ml^{-1}$ (about 6.7×10^{-7} mol l^{-1} with an average molecular weight of 15,000). Commercially available *meso*-tetrakis [(trimethylammoniumyl) phenyl] porphyrin (TAPP) and *meso*-tetra (4-methylpyridy) porphyrin (TMPyP-4) were purchased from Aldrich (St. Lewis, WO, USA), and their working solutions were 2.1×10^{-5} and 1.0×10^{-4} mol l^{-1}, respectively. Britton–Robinson buffer was used in this experiment to control pH value. All other reagents were of analytical-reagent grade without further purification or pretreatment. Millipore purified water was used throughout.

Absorption spectra were measured on a Techcomp 8500 UV–vis spectrophotometer (Hong Kong, China). RLS spectra were measured on a Hitachi F-2500 spectrofluorometer (Tokyo, Japan) by scanning simultaneously the excitation and emission monochromators of the spectrofluorometer from violet to visible region. Both the excitation and emission slits were set at 5.0 nm. An S-10A digital pH meter (Xiaoshan Scientific Instruments Company, Zhejiang, China) was used to measure the pH values. The charge distributions of porphyrins are calculated using the Hyperchem Pro 7.0 (Hypercube, USA) software in AM1 mode.

2.2. General procedure

A 1.0 ml of porphyrin solution, 1.0 ml of Britton–Robinson buffer and a serial of heparin solution were added to a 10 ml volumetric flask successively. The mixture was diluted to 10.0 ml with water and shaken thoroughly. RLS spectra were then measured on the F-2500 spectrofluorometer by simultaneously scanning the excitation and emission monochromators with same starting wavelength and same scanning velocity. The RLS ratios were obtained by dividing the RLS intensity at 442 nm with 418 nm for HP/TAPP system and that at 448 nm with 418 nm for HP/TMPyP-4 system.

3. Results and discussion

3.1. Wavelength for ratiometric measurement

Titrated with HP, the molecular absorptions of both TAPP and TMPyP-4 undergo hypochromism without significant shift of maximum wavelength, simultaneously resulting in enhanced RLS signals characterized at 437 and 452 nm maximum and at 412 and 416 nm minimum, respectively (Fig. 1). The enhanced RLS signals result from the aggregations in an absorption medium when the excitation wavelength is close to the absorption band, and could be given as following equation [5,6]:

$$I(90) = \frac{16\pi^2 a^6 n_{\mathrm{med}}^4 I_0}{r^2 \lambda_0^4} \left| \frac{m^2 - 1}{m^2 + 2} \right| \quad m = \frac{(n_{\mathrm{real}} + i n_{\mathrm{im}})}{n_{\mathrm{med}}} \qquad (1)$$

Wherein I (90) is the light scattering intensity detected at the right angle to the incident light beam, a is the size of the aggregation species, n_{med} is the refractive index of the medium, I_0 is the intensity of the incident light beam, r is the distance between the aggregation species and the detector, λ_0 is the wavelength of the incident light beam, and m is a complex index involving in the molecular absorption and the medium environments. There always is a wavelength (λ) which is close to λ_0 that makes the denominator $m^2 + 2$ in Eq. (1) equals zero, and leads up to strong enhanced values of I (90).

It is well known that visible colors and absorption spectra of chromophores are the consequence of incident light through absorption and scattering [17], and enhanced RLS signals are strongly dependent on the absorption of aggregates [5,6]. Thus, it is necessary to simultaneously consider the molecular absorption and light scattering features in order to choose appropriate wavelengths for the light scattering ratiometric measurements. Our strategy is selecting the wavelengths at the peak and the valley of UV–vis spectral ratio curve, which could be obtained by dividing UV–vis spectrum of the chromophoric component with that in the presence of the additives. Experiments showed that $\lambda_{\max}$ and $\lambda_{\min}$ in UV–vis ratio spectra are 418 and 442 nm for HP/TAPP system, and 418 and 448 nm for HP/TMPyP-4 system, respectively (Fig. 2). The wavelength of $\lambda_{\max}$ 418 nm in HP/TAPP system corresponds to the 412 nm minimum region of RLS

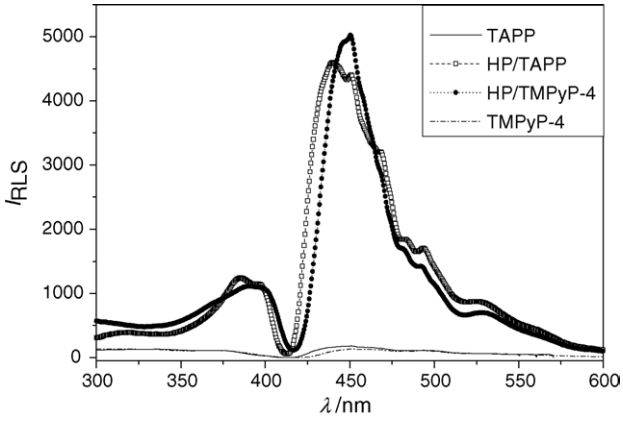

Fig. 1. Enhanced RLS signals of TAPP and TMPyP-4 by HP. c_{TAPP}, 4.2×10^{-6} mol l^{-1}; $c_{\mathrm{TMPyP\text{-}4}}$, 1.0×10^{-5} mol l^{-1}; HP, 1.5 mg ml^{-1}; pH, 7.2.