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Study on the interaction between nucleic acid and Eu^{3+}–oxolinic acid and the determination of nucleic acid using the resonance light scattering technique

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Abstract

At pH 9.75, the resonance light scattering (RLS) intensity of OA–Eu^{3+} system is greatly enhanced by nucleic acid. Based on this phenomenon, a new quantitative method for nucleic acid in aqueous solution has been developed. Under the optimum condition, the enhanced RLS is proportional to the concentration of nucleic acid in the range of 1.0×10^{-9} to 1.0×10^{-6} g/ml for herring sperm DNA, 8.0×10^{-10} to 1.0×10^{-6} g/ml for calf thymus DNA and 1.0×10^{-9} to 1.0×10^{-6} g/ml for yeast RNA, and their detection limits are 0.020, 0.011 and 0.010 ng/ml, respectively. Synthetic samples and actual samples were satisfactorily determined. In addition, the interaction mechanism between nucleic acid and OA–Eu^{3+} is also investigated.

Keywords: Nucleic acids; Oxolinic acid; Resonance light scattering

1. Introduction

The quantitative analysis for micro amounts of nucleic acids is required in many fields such as biochemistry, molecular biology, biotechnology, and medical diagnostics. Since using the intrinsic fluorescence and ultraviolet absorption of nucleic acids for their determination has been severely limited by low sensitivity and serious interference [1]. Therefore, some probes based on the interaction between nucleic acids and extrinsic reagents have been employed in the determination of nucleic acids using the spectral methods. Among these spectral methods, resonance light scattering (RLS) technique has given rise to strong interest among chemists and biochemists since Pasternack et al. pioneering work [2,3] using a common spectrofluorometer. Huang et al. first used this technique for analytical purposes to determine trace amounts of nucleic acids [4,5]. Subsequently, more studies of quantitative determination of macromolecules including nucleic acids [6–8] and proteins [9,10] and high sensitivity detection of DNA hybridization [11] by RLS have been published. Up to now, most of the probe applied in the determination of nucleic acids are organic dyes [12,13] by their aggregation on DNA, these methods used above probe are sensitive, inexpensive and safe, but they also have the disadvantage of easy to be interfered by foreign ions and narrow linear range.

In this paper, our focus is to develop the complex of Eu^{3+} and oxolinic acid (OA) as a sensitive RLS probe for the determination of trace amount of nucleic acids. OA is one of quinolone antibiotics used in treating bacterial diseases in aquatic species. Eu^{3+}–OA complex and nucleic acid have drastic interaction. The detection limit reaches 10^{-11} g/ml for nucleic acids. And its high ability to tolerance foreign ions and broad linear range also overcome the limitation on selectivity and narrow linear range of other method used dye. So study this question is important since it not only improved the

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detection limit of nucleic acid but also developed the applied range of RLS.

2. Experimental

2.1. Apparatus

The RLS spectra and the intensity of RLS were measured with a F-4500 spectrofluorimeter (Hitachi, Japan). All absorption spectra were measured on a UV-4100 spectrophotometer (Hitachi, Japan). All pH measurements were made with a Delta 320-S pH meter (Mettler Toledo).

2.2. Chemicals

Stock solutions of nucleic acids (100 μg/ml) were prepared by dissolving commercially purchased herring sperm DNA (fsDNA, Sigma, Germany), calf thymus DNA (ctDNA, Beijing Baitai Reagent Company, Beijing, China), and yeast RNA (yRNA, Sigma, Germany) in doubly deionized water. Working standard solutions were obtained by appropriate dilution of the stock solutions.

Stock standard solution of Eu^{3+} (1.0×10^{-2} mol/l) was prepared by dissolving the corresponding oxide (99.9%) in hydrochloric acid and heating until nearly dry then diluting with doubly deionized water.

Stock solution of OA (5.0×10^{-3} mol/l) was prepared by dissolving the appropriate amount of OA with 0.1 mol/l NaOH, and diluted with 3.0×10^{-3} mol/l NaOH. Working standard solution was obtained by appropriate dilution of the stock solution.

A 0.05 mol/l Tris–HCl buffer solution was prepared by dissolving of 3.03 g Tris in 500 ml volumetric flask with water and adjusted the pH to 9.75 with HCl.

All reagents were of analytical reagent grade without further purification, and doubly deionized water was used throughout.

2.3. General procedure

To a 25 ml test tube, working solutions were added in the following order: 1.0 ml of 1.0×10^{-4} mol/l Eu^{3+}; 1.0 ml of 1.0×10^{-3} mol/l OA; 1.0 ml of 0.05 mol/l Tris–HCl (pH 9.75); and appropriate amount of nucleic acid solutions. The mixture was diluted to 10 ml with doubly deionized water and shook to react efficiently and allowed to stand for 5 min. All RLS spectra were obtained by scanning simultaneously the excitation and emission monochromators (namely, $\Delta\lambda = 0$ nm) from 200 to 700 nm. The intensity of RLS was measured at $\lambda = 365$ nm in a 1 cm quartz cell with slit width at 10 nm for the excitation and emission. The enhanced RLS intensity of Eu^{3+}–OA system by nucleic acids was represented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$, here I_{RLS} and I^0_{RLS} were the intensities of the system with and without nucleic acids.

3. Results and discussion

3.1. RLS spectra and absorption spectra of Eu^{3+}–OA–DNA system

Fig. 1 shows the light scattering spectra of Eu^{3+}–OA–DNA system. Both OA and Eu^{3+}–OA show very weak RLS signals over the wavelength range of 200–700 nm. However, when Eu^{3+}–OA mixed with DNA in pH 9.75, the RLS intensity is strongly enhanced indicating an interaction between DNA and Eu^{3+}–OA. Fig. 2 is the absorption spectra of the system. It can be seen from line 1 that the absorption peak of OA located in 258, 266, 326 and 340 nm. Compare Fig. 1 with Fig. 2 according to the theory of RLS [2,14], the RLS peak at

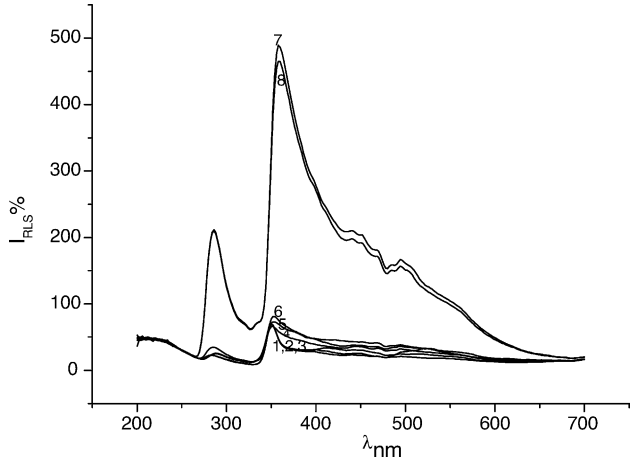

Fig. 1. Resonance light scattering spectra of the Eu^{3+}–OA–fsDNA system. (1) OA; (2) OA–Tris; (3) OA–Tris–fsDNA; (4) OA–Eu^{3+}; (5) Eu^{3+}–OA–fsDNA; (6) OA–Eu^{3+}–Tris; (7) OA–Eu^{3+}–Tris–fsDNA; (8) OA–Eu^{3+}–Tris–yRNA. Conditions: OA, 1.0×10^{-4} mol/l; Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml (pH 9.75); fsDNA, 5.0×10^{-7} g/ml; yRNA, 5.0×10^{-7} g/ml.

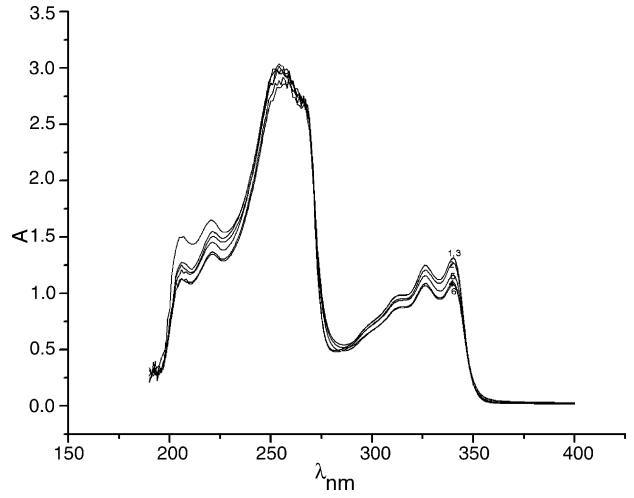

Fig. 2. Absorption spectra of Eu^{3+}–OA–fsDNA system. (1) OA; (2) OA–fsDNA; (3) OA–yRNA; (4) Eu^{3+}–OA; (5) Eu^{3+}–OA–fsDNA; (6) Eu^{3+}–OA–yRNA. Conditions: OA, 1.0×10^{-4} mol/l; Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml; fsDNA, 5.0×10^{-7} g/ml.