Study of the interaction of nucleic acid with europium(III) and CTMAB and determination of nucleic acids at nanogram levels by the second-order scattering

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Abstract

This paper firstly reports the second-order scattering (SOS) phenomenon in the nucleic acid system using a common spectrofluorometer. And a novel determination method of nucleic acids at nanogram level has been developed. Studies involving calf thymus DNA (ctDNA), fish sperm DNA (fsDNA) and yeast RNA (yRNA) showed that the SOS intensity of nucleic acids can be enhanced by La^{3+}, Ce^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, Gd^{3+} and Tb^{3+} ion, of which Eu^{3+} ion has the largest enhancement. And the intensity of the SOS is proportional to the concentration of nucleic acids. Maximum SOS peak at 550 nm appeared at pH 7.5 and $6.0 \times 10^{-5} \, mol \, l^{-1}$ CTMAB (cetyltrimethylammonium bromide). Under the optimal conditions, the calibration graphs were linear in the range of 1.0×10^{-8} to $4.0 \times 10^{-5} \, g \, ml^{-1}$ for ctDNA, 6.0×10^{-9} to $2.0 \times 10^{-6} \, g \, ml^{-1}$ for fsDNA, and 8.0×10^{-9} to $1.0 \times 10^{-6} \, g \, ml^{-1}$ for yRNA, respectively. The detection limits were $1.7 \, ng \, ml^{-1}$ for ctDNA, $0.36 \, ng \, ml^{-1}$ for fsDNA and $0.21 \, ng \, ml^{-1}$ for yRNA, respectively. In comparison with most other methods for the determination of nucleic acids, this method is more convenient, more sensitive and simpler. And the possible mechanism was proposed.
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1. Introduction

Nucleic acids have an important function in life processes, thus research on them has become a significant element in life sciences. The quantitative determination of nucleic acids is the basis for the study of nucleic acids, which can be used as a reference for measurement of other components in biological samples, and is very important in biochemistry and molecular biology [1,2]. The determination methods for nucleic acids are well reported. But because of the low fluorescence quantum yield of native nucleic acids, direct use of the fluorescence properties of nucleic acids to study the biological properties is limited [3–5]. Therefore, a number of extrinsic fluorescence probes have been employed for the

determination of nucleic acids. The fluorescence probes include: organic dyes such as ethidium bromide (EB) [6–10], diaminophenylindole (DAPI) [11,12], bisimidazole (Hoechst 33258) [13–15], Magdala red [16], thiazole orange homodimer (TOTO) and oxazide yellow homodimers (YOYO) [17], metal complexes, and metal ions [18,19], especially the lanthanide(III) cations. The trivalent lanthanide cation is very sensitive to environment and easily quantified when combining with nucleic acids, such as Tb(III) [20–24] and Eu(III) [25].

Light scattering is a common phenomenon and has a wide application. In 1993, Pasternack et al. developed the resonance light scattering (RLS) technique by using a common spectrofluorometer [26,27]. In recent years, RLS as a basis of a new technique has been applied to determine biological macromolecules, such as nucleic acids [28,29] and proteins [30], trace amounts of inorganic ions [31–33], and cation

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surfactant [34]. Based on the macroscopic fluctuation theory [35] and Mie theory [36,37], the intensity of scattered Rayleigh light is in proportion to λ_0^{-4}. In fact, the RLS spectrum is a particular synchronous scan spectrum at $\Delta\lambda = 0$ nm (i.e. $\lambda_{ex} = \lambda_{em}$). On the otherhand, it is well known that there is often a peak at the double excitation wavelength ($2\lambda_{ex}$) named the second-order scattering [38] in the fluorimetric determination which is regarded as an interfere peak and usually eliminated. Actually, the SOS spectrum is an emission spectrum. Liu et al. first studied SOS as an analytical technique in 1995 [39,40], and successfully detected the trace amount of selenium [41], mercury [42,43], chromium [44], cadmium [45], heparin [46], cationic surfactant [47] and β-cyclodextrin [48] with the SOS technique. However, the SOS phenomenon of the nucleic acids system and the determination of nucleic acids with SOS technique have not been reported.

In this paper, the second-order scattering (SOS) of Eu(III)–CTMAB–nucleic acid system is reported. The results showed that the SOS intensity of Eu(III)–CTMAB–nucleic acid system is much larger than the system without nucleic acids and that the relative intensity is proportional to the concentration of nucleic acids. Nucleic acid at nanogram level is successfully determined. The mechanism of interaction of nucleic acids with Eu(III) ion and CTMAB is proposed.

2. Experimental

2.1. Reagents

Stock solutions of nucleic acids ctDNA (Sigma), fsDNA (Sigma) and yRNA (Sigma) were prepared by dissolving in doubly distilled water and diluted to the final concentration of 500 μg ml^{-1}, respectively. Twenty-four hours or more were needed for complete dissolution of nucleic acids with gentle shaking. The molarities of nucleic acids were calculated according to the absorbance at 260 nm by using molar extinction coefficient ε_{DNA} (6600 mol^{-1} cm^{-1}) and ε_{RNA} (7800 mol^{-1} cm^{-1}) after establishing that the absorbance ratio A_{260}/A_{280} was 1.8 for DNA and 2.0 for RNA, respectively [49].

The standard stock solution of the Eu(III) ion (0.01 mol l^{-1}) was prepared by dissolving 352 mg Eu$_2$O$_3$ in 10 ml HNO$_3$ (15 mol l^{-1}) at 100 °C and evaporating the solution to be almost dry, and then diluting it to 500 ml with water. The stock solutions of other lanthanide(III) ions were prepared similarly.

A stock solution of cation surfactant, cetyltrimethylammonium bromide (CTMAB, 1.0×10^{-2} mol l^{-1}) was prepared by dissolving 364.5 mg CTMAB in 100 ml warm water.

Tris–HCl buffer solution (0.05 mol l^{-1}, pH = 7.5) was used to control the acidity of the solution. Tris–HCl buffer solution (0.05 mol l^{-1}) was prepared by dissolving 3.03 g of tris-(hydroxymethyl)aminomethane in 250 ml water and adjusting pH to 7.5 with 0.1 mol l^{-1} HCl.

All chemicals were of analytical grade and doubly distilled water was used throughout. All stock solutions were stored at 0–4 °C.

2.2. Apparatus

The SOS spectrum and the intensity were measured with a RF-5301PC spectrofluorometer (SHIMADU, Japan). All absorption spectra were recorded with a Cintra 10e UV–vis spectrophotometer (GBC). A 420Aplus pH Meter (Orion Research Inc.) was used to measure the pH of the solutions.

2.3. Preparation of the synthetic and real samples

According to the interferences of the coexisting substances, synthetic samples were made by adding the coexisting substances in an appropriate volume of the standard solution.

The real sample of yeast RNA was prepared by the following described protocol: yeasts were grown in stationary phase at 37 °C, concentrated by centrifugation and washed twice by distilled water. The yeasts were ground and transferred to a tube with the ice TRIZOL reagents (monophasic solutions of phenol and guanidine isothiocyanate for the isolation of total RNA from cells and tissues) (Invitrogen), and 0.2 ml chloroform was added. The mixture was centrifuged at 12,000 rpm for 15 min at 4 °C. The liquid phase was collected in a new centrifuge tube, and 0.5 ml 2-propanol was added. Finally yRNA was collected by centrifugation at 12,000 rpm for 10 min at 4 °C, and washed sequentially with 80 and 100% (v/v) ethanol, air dried and then dissolved in nuclease-free water [50].

2.4. Procedure

Solutions were added in a tube in order of Tris–HCl, CTMAB, DNA, Eu(III) ion and then diluted to 10 ml with water, mixed thoroughly and stood for 30 min. All the SOS intensities (ΔI_{SOS}) were measured against the blank which is prepared by the same way, but without DNA, and thus $\Delta I_{SOS} = I_{SOS} - I_{SOS}^0$ is obtained. The SOS spectrum was reported with $\lambda_{em} = 2\lambda_{ex}$ from 290 to 900 nm. All data were obtained by using the slit-width of the excitation and the emission of the spectrofluorometer at 5 nm.

3. Results and discussion

3.1. SOS and UV–vis spectra

The SOS peak locates at the site of the $2\lambda_{ex}$. It can be found that the maximum SOS peak is at 550 nm by changing the wavelength of incident light with the exciting light at 275 nm in the system of Eu(III)–nucleic acids–CTMAB. In order to distinctly observe the SOS spectra, the high sensitivity was used in recording the SOS spectra. Fig. 1 shows the