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Resonance light scattering spectroscopy of procyanidin–CPB–DNA ternary system and its potential application

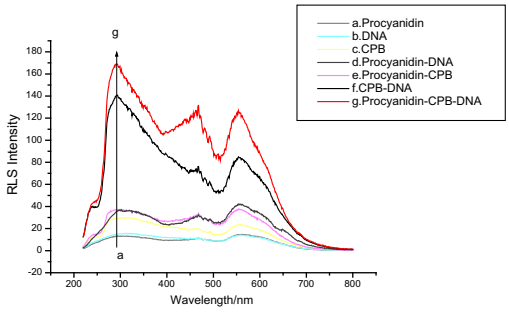
Yu Wang, Shuyun Bi *, Huifeng Zhou, Tingting Zhao

College of Chemistry, Changchun Normal University, Changchun 130032, PR China

HIGHLIGHTS

- A new RLS system: procyanidin–CPB–DNA was investigated.
- Linear range for DNA determination is 0.0084–3.36 μg mL^{-1}.
- DNA detection limit is 2.27 ng mL^{-1}.

GRAPHICAL ABSTRACT



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ABSTRACT

A new method for the determination of calf thymus DNA at nanogram level was proposed based on the enhanced resonance light scattering (RLS) signals of DNA in the presence of procyanidin and cetylpyridinium bromide dihydrate (CPB). Under the experimental conditions, the RLS intensity of DNA at 291.0 nm was greatly enhanced by procyanidin–CPB at pH 7.0. There was a good linear relationship ($r = 0.9993$) between the enhanced RLS intensity (ΔI_{RLS}) and DNA concentration of 0.0084–3.36 μg mL^{-1}. The limit of detection (LOD) was 2.27 ng mL^{-1} ($3S_0/S$). Three synthetic DNA samples were measured with satisfactory, and the recovery was 102.3–107.2%.

Introduction

The research done by Pasternack and his co-workers has shown resonance light scattering (RLS) is a valuable technique for detecting and characterizing extended aggregates of chromophores [1–6]. The scattering intensity of species is enhanced by several orders of magnitude at or near the wavelength of absorption when electronic coupling exists among the chromophore units [7]. As a result, RLS is an extremely sensitive technique for monitoring molecular assemblies. For analytical purpose, RLS technique was widely used for the determination of nucleic acids [8–11], proteins [12–14], medicine [15,16], etc. DNA analysis plays an important role in a wide range of areas including the diagnostics of genetic diseases, the monitoring of infectious bacteria, the analysis of forensic samples, and the screen of bioterrorism agents [17]. The determination of concentration of DNA is an important aspect of DNA analysis. Most of the studies were mainly based on the fact that the assemblies of various probes [18–20] on DNA gave rise to enhanced RLS. Considerable effort has been made to find natural and non-toxic RLS probes. In this study, procyanidin (Fig. 1) was introduced as a new probe for the RLS determination of DNA with cetylpyridinium bromide (CPB), which has not been reported to our knowledge. Procyanidins are a group of monomers and polymers of flavonols (without added sugars) [21], which are internationally recognized as the most effective natural antioxidants.

* Corresponding author. Tel.: +86 431 86168098; fax: +86 431 86168096.
E-mail address: sy_bi@sina.com (S. Bi).

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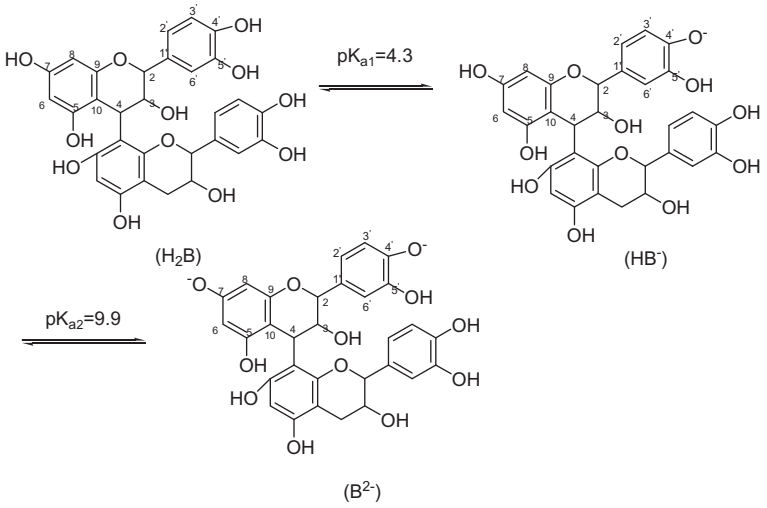

Fig. 1. Structure of procyanidin and the dissociation equilibrium.

Here, CPB, a common cationic surfactant, was chosen as another crucial part of constituting ternary complex of procyanidin–CPB–DNA. It was found the weak RLS signal of DNA could be enhanced drastically by procyanidin–CPB. As we know, CPB can interact with phosphate groups of DNA by electrostatic attraction. At pH 7.0, the predominant form of procyanidins is a deprotonated form (see below, the pK_a of procyanidin gained from this assay would contribute to the studies on procyanidin in the future), which is negatively charged. The RLS signal of DNA could not be changed by procyanidin alone. However, the introducing of CPB to the binary system (procyanidin–DNA) greatly enhanced the signal. Under optimum conditions, the increment of RLS signals is proportional to the concentration of DNA. So, a novel assay was successfully developed and applied to the determination of DNA. Compared with the existing DNA assays, the expenditure, simple measurement and the lower detection limit were superior.

Experimental

Materials and reagents

Stock solution of DNA (160 μg mL^{-1}) was prepared by dissolving commercially purchased calf thymus DNA (ctDNA, Sigma Chem. Co.) in 100 mL double distilled water overnight. The DNA stock solution was stored at 0–4 °C. The concentration of DNA was calculated according to the A_{260} with molar absorptivity of 6600 L mol^{-1} cm^{-1}. The purity of DNA was checked by monitoring the ratio of the absorbance at 260–280 nm. A qualitative check was provided by the A_{260}/A_{280} ratio, which usually exceeded 1.80 [22].

Procyanidin (Chinese Drug Biological Products Qualifying Institute) was prepared to be a 1.0×10^{-4} mol L^{-1} stock solution by dissolving 0.0059 g in the 100 mL volumetric flask in water. CPB (Beijing Chemical Reagent Co., Ltd.) was prepared to be a 1.0×10^{-3} mol L^{-1} aqueous solution.

Tris–HCl buffer solution (containing 0.1 mol L^{-1} NaCl) was used to remain the pH value.

All chemicals used were of analytical reagent grade and the double distilled water was used all along.

Apparatus

The RLS spectra were measured by a RF-5301PC spectrofluorometer (Shimadzu, Japan) equipped with a xenon lamp source and a quartz cuvette (1.0 cm × 1.0 cm × 4.0 cm). A TU-1901 spectrometer (Beijing Purkinje General Instrument Co., Ltd.) was used to acquire UV–vis spectra.

Experimental procedures

Procyanidin and CPB were accurately added to a colorimetric tube, and the solution were diluted to 5 mL by appropriate amounts of Tris–HCl (pH = 7.0) buffer. After 10 min, the mixture was mixed thoroughly. For this system, the RLS intensity was measured at the maximum wavelength of 291 nm. The RLS intensity increment of procyanidin–CPB–DNA was represented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$, where I_{RLS} and I^0_{RLS} were the intensities of procyanidin–CPB with and without DNA respectively.

The RLS spectrum was obtained by scanning simultaneously the excitation and emission monochromators ($\lambda_{ex} = \lambda_{em}$) from 220.0 to 800.0 nm, both slit widths of excitation and emission were 5 nm.

Results and discussion

Spectral characteristics

As we know, if the wavelength of the incident beam is close to that of the absorption band of molecular particles that exist as aggregates, the Rayleigh scattering intensity of some wavelength will rapidly increase [1,3,23]. Hepel et al. presented the relationship of paritle diameter and RLS intensity [24,25], $\frac{I_2}{I_1} = a^3_{rel}$, where a_{rel} is the relative diameter of scattering particles, $a_{rel} = a_2/a_1$, a_1 and a_2 are the diameters of the particles before and after aggregation, I_1 and I_2 represent the RLS intensity of before and after aggregation respectively. The equation shows that a very minute change of the size of scattering particle can produce a significantly change in RLS intensity. Under the optimal conditions, the RLS spectra of procyanidin, DNA, CPB, procyanidin–DNA, procyanidin–CPB, CPB–DNA, and procyanidin–CPB–DNA were measured. As displayed in Fig. 2, the RLS signals for procyanidin alone, DNA alone, CPB alone, procyanidin–DNA and procyanidin–CPB were all weak in the whole scanning range. However, the RLS signal of CPB–DNA was much stronger. The strongest RLS signal was obtained from procyanidin–CPB–DNA system. For the CPB–DNA and procyanidin–CPB–DNA system, the maximum scattering peak was at 291.0 nm, which was therefore selected as analytical wavelength for the further study. The corresponding relationship between RLS spectra and absorption spectra could be observed from