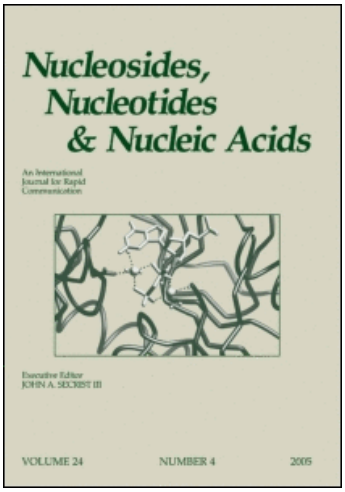

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Nianqin Jie[a]; Shicong Hou[a]; Fengpei Du[a]; Chunrong Zhang[a]; Guoxin Qin[a]
[a] College of Applied Chemistry, China Agricultural University, Beijing, P.R. China

Online publication date: 05 June 2004

To cite this Article Jie, Nianqin , Hou, Shicong , Du, Fengpei , Zhang, Chunrong and Qin, Guoxin(2004) 'The Determination of Deoxyribonucleic Acids with Triadimenol Based on the Enhancement of Resonance Light Scattering', Nucleosides, Nucleotides and Nucleic Acids, 23: 4, 725 — 734
To link to this Article: DOI: 10.1081/NCN-120037750
URL: http://dx.doi.org/10.1081/NCN-120037750

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, No. 4, pp. 725–734, 2004

The Determination of Deoxyribonucleic Acids with Triadimenol Based on the Enhancement of Resonance Light Scattering

Nianqin Jie,* Shicong Hou, Fengpei Du, Chunrong Zhang, and Guoxin Qin

College of Applied Chemistry, China Agricultural University, Beijing, P.R. China

ABSTRACT

For the first time, triadimenol was used to determine nucleic acid (DNA) using the resonance light scattering (RLS) technique. The RLS of triadimenol was greatly enhanced by DNA in the range of pH 1.6 $\sim$ 1.9. A resonance light-scattering peak at 310nm was found, and the enhanced intensity of RLS at this wavelength was proportional to the concentration of DNA. The linear range of the calibration curve was 0 $\sim$ 9 μg/ml with the detection limit of 24 ng ml^{-1}. The mechanism studies of the system indicated that the enhanced RLS is due to the aggregation of triadimenol on DNA. The nucleic acids in synthetic samples and in rice seedling extraction were

*Correspondence: Nianqin Jie, College of Applied Chemistry, China Agricultural University, Beijing 100094, P.R. China; E-mail: aydy-826@163.com.

DOI: 10.1081/NCN-120037750 1525-7770 (Print); 1532-2335 (Online)

analyzed with satisfactory results. Compared with other methods, this method is convenient, rapid, inexpensive and simple.

Key Words: Deoxyribonucleic acids; Triadimenol; Resonance light scattering.

INTRODUCTION

Nucleic acids have an important function in life processes. They are the carrier of genetic information. The quantitative determination of nucleic acids is of great importance in fundamental research and in clinical diagnosis. However, it is difficult to detect nucleic acids by using their native fluorescence because of the poor fluorescence quantum efficiency ($\Phi = 1.0 \times 10^{-5}$),[1] and, therefore, extrinsic fluorescent probes such as organic small molecular reagents,[2] metal ions,[3,4] and metal complexes[5–7] are usually introduced in studies concerning nucleic acids.

Rayleigh scattering is a kind of light scattering phenomenon. The technique suffers from the disadvantages of low sensitivity and selectivity. Pasternack et al.[8,9] found that resonance light scattering (RLS) can be observed in porphyrin-DNA complexes when the wavelength of the incident light is near an absorption band of porphyrin. This confirmed that porphyrin associates with DNA.

The resonance light scattering of associated molecules can be detected, even using a common spectrofluorimeter. Hung et al.[10,11] applied resonance light scattering technique to analyze protein and nucleic acids. From then on, the resonance light-scattering technique, no addition further followed spectrophotometric method[12] and the fluorescence method[13] has become very popular for the determination of biological macromolecules. Subsequently, more studies of the quantitative determination of macromolecules including nucleic acids and proteins by RLS have been reported. Safranine T,[14] phenosafranine,[15] rhodamine B,[16] nile blue aulphate,[17] ethyl violet,[18] brilliant crystal blue,[19] neutral red,[20] crystal violet[21] and resaniline[22] were successfully used to determine nucleic acid concentration.

In this article, triadimenol was used as a probe for the determination of nucleic acids using the resonance light scattering. Triadimenol is an insecticide and its structure is shown in Fig. 1. To our knowledge, however, the use of triadimenol as a probe for the determination of nucleic acids has not been reported so far. For the first time we show that the insecticide triadimenol can also give strong RLS signals in the presence of DNA. Based on this reaction, a new method of determining nucleic acids was developed with a common spectrofluorimeter and inexpensive reagents. The method leads to a particularly inexpensive, simple and sensitive system, permitting a limit of

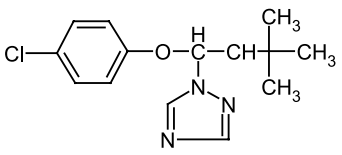

Figure 1. Structure of triadimenol.

detection of 24 ng ml^{-1} DNA. The mechanism of the reaction between triadimenol and DNA is also discussed.

EXPERIMENTAL

Apparatus

All resonance light-scattering measurements were measured with a Hitachi F-4500 fluorescence spectrophotometer (Japan) with a 150W Xe lamp and a 1-cm quartz cell. The pH measurements were made with a model pHS-3C pH meter (Shanghai, China).

Reagents

All chemicals used were of analytical reagent grade or the highest available purity. All aqueous solutions of the reagents were made up in distilled water that had been processed with an ion-exchange resin.

Stock solutions of nucleic acids (100 μg ml^{-1}) were prepared by dissolving commercial calf thymus DNA (Baitai, Beijing, China) in deionized water and stored at 4°C. The solution was diluted to 10.0 μg ml^{-1} with water as working solution. Triadimenol solution (100 μg ml^{-1}) was prepared by dissolving 10 mg of triadimenol (China Agricultural University)in 0.5 ml isopropyl alcohol and then diluted to 100 ml with deionized water. Hydrochloric acid solution: 0.1 mol L^{-1}. A Tris-HCl buffer solution (pH 7.0), including 50 mM Tris-HCl , 100 mM NaCl, 50 mM EDTA, 0.2% sodium dodecyl sulfate (SDS), and a TNE buffer including 10 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA were used for the extraction of nucleic acid from rice seedlings.

Procedure

To a 10-ml standard flask, solutions were added in the following order: 1.0 ml of triadimenol (100 μg ml^{-1}), a known volume of ctDNA standard solution, 2.6 ml of HCl solution. The solution was then diluted to the mark with water and mixed thoroughly. All RLS measurements were obtained relative to a blank prepared in the same way but without nucleic acids.

The RLS spectra were obtained by scanned synchronously the excitation and emission monochromators ($\lambda ex = \lambda em$) through the wavelength range 250–600 nm. All data were obtained with 5.0 nm excitation and emission slit-widths. The RLS intensity was routinely measured at the maximum wavelength, 310 nm.

RESULTS AND DISCUSSION

Resonance Light Scattering Spectra

Figure 2 shows the resonance light-scattering spectra of triadimenol, calf thymus DNA and their mixture. The figure shows that the RLS intensity of triadimenol and calf

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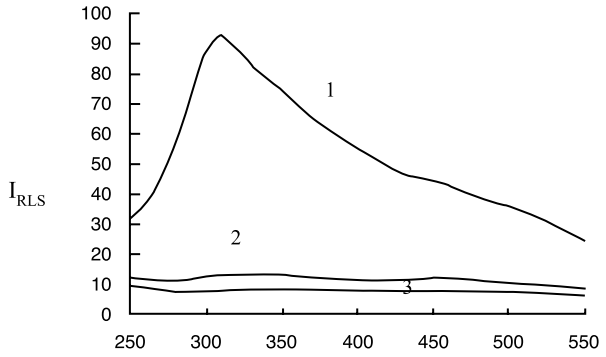

Figure 2. Resonance light-scattering spectra of triadimenol-deoxyribonucleic acid system. Concentration: pH = 1.7; triadimenol, 3.4×10^{-4} mol L^{-1}, DNA; 1.0 μg/ml.

thymus DNA is weak when they exist separately. When DNA and triadimenol coexisted, however, the intensity of resonance light scattering was strongly enhanced in the wavelength range of 300 ~ 500 nm,and the enhanced peaks could be observed at 310 nm. Therefore, the optimal wavelength for the resonance light scattering measurements of DNA was chosen to be 310 nm.

The Optimized Reaction Conditions

Effect of pH

It can be seen from Fig. 3 that pH influences the value of the resonance light-scattering (RLS) intensity of the mixture of calf thymus DNA and triadimenol

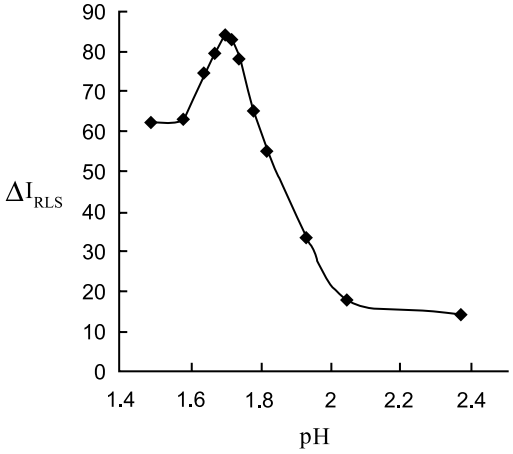

Figure 3. The effect of pH on the intensity of RLS. Concentrations: triadimenol, 10 μg ml^{-1}; DNA 1.0 μg ml^{-1}.

significantly. An HCl solution was used to adjust the pH. As shown in Fig. 3, first, the RLS of the system increased with increasing HCl concentration and then decreased. The maximum values of the RLS intensity occur in the pH range of 1.6–1.9. Since the intensity at pH 1.7 is the highest, the optimal pH is chosen as 1.7 in this paper.

The Optimal Concentration of Triadimenol

The optimal concentration of triadimenol was examined for ctDNA (1.0 μg ml^{-1}) by varying the concentration of triadimenol from 1.7×10^{-4}–1.5×10^{-3} mol L^{-1} at pH 1.7. With the change in the concentration of triadimenol, the intensity difference values ΔI_{RLS} between the system with or without DNA through change in the concentration of triadimenol show that the slight influence of the concentration of triadimenol on ΔI_{RLS} was observed. A 3.4×10^{-4} mol L^{-1} triadimenol solution was chosen for further study.

Stability Test

Under the optimal condition, the effect of time on the resonance light scattering intensity of the ctDNA and triadimenol system was tested when the concentration of ctDNA was 1.0 μg ml^{-1}. The results showed that resonance light scattering signal reached a constant value within 3 min after triadimenol had been added and remained stable up to 40 min.

Calibration

The calibration graphs for the determination of DNA were constructed. Under optimal conditions, the ratio value of resonance light scattering intensities of triadimenol in the presence of nucleic acids was proportional to the concentration of nucleic acids in a good linear relationship. A calibration graph for a series of standard solution of DNA ($0 \sim 9$ μg ml^{-1}) provided a typical calibration line with the following analytical regression feature: $\Delta I_{RLS} = 18.48C + 4.44$ (C:μg ml^{-1}). The regression coefficient was r = 0.9857. The determination limit, corresponding to signal to noise ratio of 3 was 0.024μg ml^{-1}. The relative standard deviation for five replicate measurements of 1μg ml^{-1} DNA was 1.3%.

Interferences of Foreign Substances

The influence of some common ions and bases on the RLS assay for nucleic acids was investigated at pH 1.7. The results were listed in Table 1. It can be seen that HPO_4^{2-}, K^+ and $H_2PO_4^-$, Na^+ ions scarcely cause the interference. Although the other ions and substances tested can be tolerated at relatively low levels, their quantities in biological samples diluted for analysis are usually below the amounts tolerated under experimental conditions.

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Table 1. Interference of foreign substances concentrations: triadimenol: 3.4×10^{-4} mol/L; DNA: 1.0 μg/ml; pH 1.7.

Foreign substance	Co-existing concentration	Relative error (%)
Adenine (A)	0.5 μg L^{-1}	2.3
Guanine (G)	0.25 μg L^{-1}	− 4.0
$H_2PO_4^-$, Na^+	3×10^{-5} mol L^{-1}	− 4.5
Fe^{3+}, Cl^-	1×10^{-7} mol L^{-1}	− 3.4
Cu^{2+}, SO_4^{2-}	8×10^{-7} mol L^{-1}	− 2.6
Mn^{2+}, SO_4^{2-}	4×10^{-7} mol L^{-1}	− 2.8
Cr^{3+}, Cl^-	4×10^{-7} mol L^{-1}	5.0
Ca^{2+}, Cl^-	1×10^{-8} mol L^{-1}	3.3
Zn^{2+}, SO_4^{2-}	8×10^{-7} mol L^{-1}	3.0
Ni^{2+}, SO_4^{2-}	2×10^{-7} mol L^{-1}	3.7
Pb^{2+}, NO_3^-	1×10^{-8} mol L^{-1}	4.8
Cd^{2+}, SO_4^{2-}	8×10^{-8} mol L^{-1}	4.0
Hg^{2+}, Cl^-	2×10^{-7} mol L^{-1}	− 2.5
Co^{2+}, Cl_-	3×10^{-7} mol L^{-1}	3.8
HPO_4^{2-}, K^+	1×10^{-5} mol L^{-1}	− 4.2

Comparison of the Methods with Other Methods

Some characteristics of the proposed method and other methods of RLS for nucleic acid determination were compared in Table 2. It can be seen that all the RLS methods require almost no incubation time. At the same time, Table 2 indicates that the proposed method has a higher sensitivity and its linear range is wider than other methods.

Sample Analysis

The Determination of Nucleic Acids in Synthetic Samples

Three samples of ctDNA containing metal ions, bases, etc., based on the tolerance of co-existing species, were analyzed. The results, listed in Table 3, indicate that the results of determination of DNA in synthetic samples are satisfactory.

Table 2. Comparison of method of RLS for determination of DNA.

Method	Incubation time (min)	LOD (mg ml^{-1})	Linear range (μg ml^{-1}
Crystal violet[21]	< 2	36.8	0–4.0
Brilliant crystal blue[20]		23.3	0.08–1.0
Neutral red[21]		128	0–0.6
Resaniline[22]		14.2	0–1.0
Phenosafranine[16]		3.2	0.5–4.0
Ethyl violet[19]		1.54	0–0.5
Safranine T[15]	< 2	13.2	0–2.5
This method	3	24	0–9.0

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Table 3. Determination of DNA in synthetic samples.

DNA content in samples (μg/ml)	Main interference	Found μg/ml, n = 5	Recovery (%)	RSD (%) (n = 5)
2.00	$H_2PO_4^-$ (6.25×10^{-7} mol L^{-1}) Ca^{2+}, Mg^{2+}(2.5×10^{-9} mol L^{-1}) Adenine, Guanine (0.25 μg ml^{-1})	1.96	98.0	0.6
2.00	$H_2PO_4^-$ (1.25×10^{-6} mol L^{-1}) Ca^{2+}, Mg^{2+}(5×10^{-9} mol L^{-1}) Adenine, Guanine (0.5 μg ml^{-1})	1.97	98.5	2.0
2.00	$H_2PO_4^-$ (2.5×10^{-6} mol L^{-1}) Ca^{2+}, Mg^{2+}(1.0×10^{-8} mol L^{-1}) Adenine, Guanine (1.0 μg ml^{-1})	2.08	104.0	0.4

The Determination of Nucleic Acids in Rice Seedlings Extraction and Recovery Test

The isolation of nucleic acid from rice seedlings was very rapid. In this procedure, 4 g of dry rice seedlings were ground into powder with liquid N_2 and were transferred to a centrifuge tube. DNA obtained from rice seedlings by extraction according to the method reported by Isao Karube[23] was determined. As an example of further use of the method, a recovery test of DNA in rice seedlings extraction was carried out. To three same volume diluted samples of rice seedlings extraction, a known amount of ctDNA was added successively to each sample to a level of $1 \sim 3$ μg ml^{-1}, and the DNA concentration was determined with the system after each addition. The analytical results, summarized in Table 4 and Table 5, show that the proposed method is acceptable for the determination of DNA in rice seedling extraction.

Table 4. The results of the recovery of DNA in rice seedlings extraction.

Standard added (μg ml^{-1})	Found value (μg ml^{-1})	Recovery (%)	RSD (%) (n = 5)
1.00	1.05	105.0	2.3
2.00	2.10	105.0	4.4
3.00	3.05	101.6	3.2

Table 5. Determination of DNA in rice seedling solution.

Volume of rice seedling solution (ml)	Found value ($\mu g\ ml^{-1}$)	RSD (%)	DNA content in rice seedlings ($\mu g\ g^{-1}$)
2.00	0.485	3.3	40.4

Mechanism of the Reaction Between Triadimenol and DNA

Electrostatic Effect

In the acidic medium, triadimenol became positively charged while nucleic acids are negatively charged and their negative electric charges were centralized on the phosphate of the nucleic acid framework. Figure 2 shows that the RLS intensity of triadimenol and calf thymus DNA is weak when they exist separately. When DNA and acetamiprid coexisted, however, the RLS intensity was strongly enhanced. It indicated that triadimenol reacted with nucleic acids to form an ion-association complex, which could cause enhanced RLS of triadimenol.

Cumulative Effect

Triadimenol contains a planar conjugated heterocyclic, so a π-π interaction is produced between triadimenol and nucleic acid base. It is shown that triadimenol interacts with nucleic acid bases to form a π-π cumulative effect.

In addition, hydrophobic effect and hydrogen bond between triadimenol and DNA are possibly present. They could also enhance RSL.

CONCLUSIONS

A sensitive and convenient method for the determination of nucleic acids was established. Resonance light scattering was applied in the method and the RLS intensity showed a good linear relationship with nucleic acid concentration. The lower limit of detection was 24 ng ml^{-1} for DNA, and its application to the determination of DNA extracted from rice seedlings was satisfactory. The performance of the system indicated that it could be used as a rapid method for DNA in biological samples. The mechanism of the interaction between DNA and triadimenol possibly include both electrostatic effects and cumulative effects. The aggregation of triadimenol on DNA bases could cause enhanced RLS of triadimenol. Further research for the mechanism is ongoing in our laboratory.

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Received May 7, 2003
Accepted January 6, 2004

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