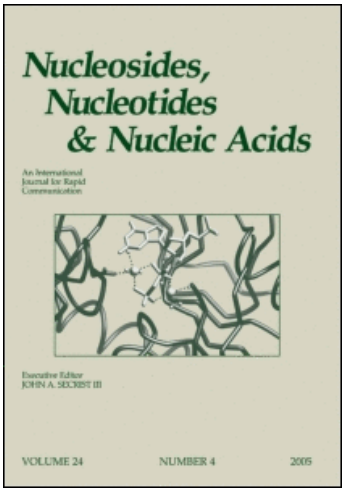

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Study on the Interaction Between Nucleic Acids and Acetamiprid

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ABSTRACT

The interaction between deoxyribonucleic acid (DNA) and acetamiprid was studied. It was found that the fluorescence of acetamiprid could be enhanced in the presence of DNA in sulfuric acid solution. The excitation and emission wavelength of acetamiprid was 291 nm and 587 nm, respectively. Under optimal conditions, the calibration graph is over the range of 0.1–10 $\mu g\,mL^{-1}$. The calibration limit is 0.06 $\mu g\,mL^{-1}$ ($S/N = 3$). The determination results of DNA in yeast cell and golden staphylococcus samples by this method were satisfactory. The mechanism of the reaction is discussed.

Key Words: Nucleic acid; Acetamiprid; Yeast; Golden staphylococcus.

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INTRODUCTION

Nucleic acids have an important function in life processes; therefore the quantitative analysis of nucleic acids is of interest. Because the fluorescence quantum yield of native DNA is low (4×10^{-5}), the direct fluorimetric determination of DNA has been limited.[1,2] Such studies on nucleic acids have been directed toward the fluorescence probes including organic dyes,[3–7] metal complex,[8–10] and light.[11] However, the main disadvantage of these methods is that lanthanide metals (such as Eu^{3+} and Tb^{3+}) and cyanine dyes (such as TOTO, YOYO, etc) are very expensive. In this article, we report acetamiprid as a fluorescence probe for the determination of nucleic acids. Acetamiprid is a new insecticide and displays weak fluorescence emission at 587 nm with maximum excitation at 291 nm. The structure of acetamiprid is shown in Fig. 1. To our knowledge, however, the use of acetamiprid as a fluorescence probe for the determination of nucleic acids has not been reported so far. Acetamiprid emits at long wavelength with a large Stokes shift and its fluorescence is significantly enhanced in the presence of nucleic acids in sulfuric acid medium. Therefore, acetamiprid has been employed as a fluorescence probe and a sensitive fluorimetric method has been developed for the determination of nucleic acids. The method leads to a particularly inexpensive, simple and sensitive system, permitting a limit of detection of 60 ng mL^{-1} DNA. The mechanism of the interaction between acetamiprid and DNA is also discussed.

EXPERIMENTAL

Apparatus and Reagents

All fluorescence measurements were made with a Hitachi 4500 spectrofluorimeter. All chemicals were of analytical reagent grade. All aqueous solutions were made with distilled, deionized water.

Stock solution of nucleic acids (100 μg mL^{-1}) were prepared by dissolving commercial calf thymus DNA (Beijing Baitai) in water and stored at 40°C. The solution was diluted to 1.0 μg mL^{-1} with water as working solution. Acetamiprid solution (4.0×10^{-4} mol L^{-1}) was directly dissolved 8.8 mg of acetamiprid (China Agricultural University) in 100 mL water. Sulfuric acid solution: 0.2 mol L^{-1}.

Procedure

To a 10 mL standard flask solutions were added in the following order: 2 mL of acetamiprid solution (4.0×10^{-4} mol L^{-1}), a known volume of ctDNA standard

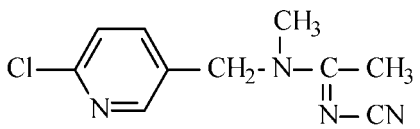

Figure 1. Structure of acetamiprid.

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solution, 0.8 mL of sulfuric acid solution. Dilute to the mark with water and mix. Measure the relative fluorescence intensity at 587 nm with excitation 291 nm in a 1 cm-quartz cell. The excitation and emission slits were both 10 nm. Background fluorescence has been subtracted for each value reported except for excitation and emission spectra.

RESULTS AND DISCUSSION

Fluorescence Spectra

The uncorrected excitation and emission spectra of DNA and DNA-acetamiprid systems were shown in Fig. 2. It was found that the excitation and emission maximum of the DNA-acetamiprid system are similar to that of acetamiprid, but the fluorescence intensity (F) significantly enhanced by adding DNA. In this paper the maximum excitation peak at 291 nm and emission peak at 587 nm were used for fluorescence intensity measurements. As can be seen, the fluorescence spectra maximum remains unchanged at 587 nm. This result is in contradistinction to the observed large red shifts in fluorescent maximum when the compounds intercalate into helix.[12] This suggests that acemiprid binds to the double helix in a non-intercalative way.

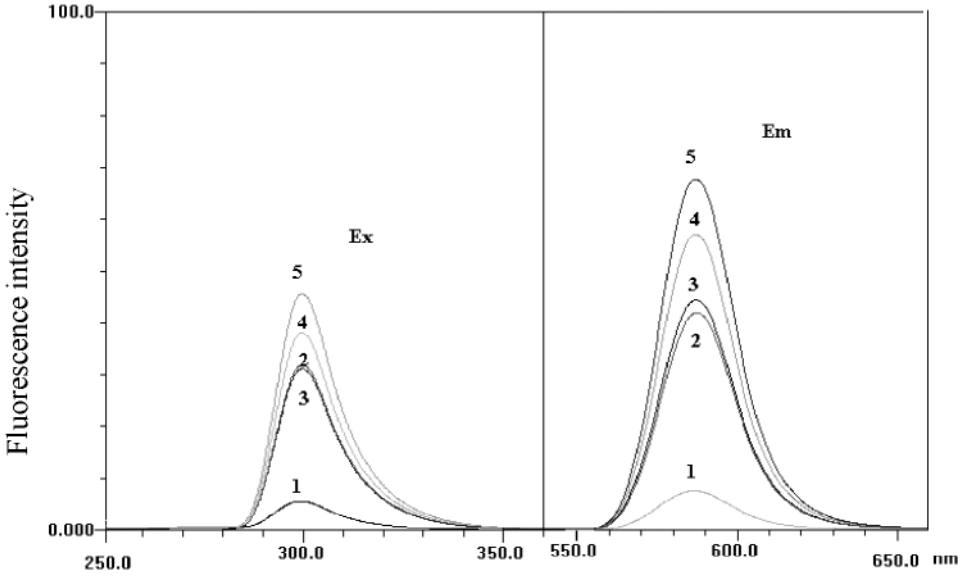

Figure 2. Excitation and emission spectra of nucleic acids- acetamiprid system. 1. acetamiprid; 2. dsDNA; 3. ssDNA; 4. acetamiprid + ssDNA; 5. acetamiprid + dsDNA C(acetamiprid): 8.0×10^{-5} mol/L; C(ctDNA): 5 mg/L; V(H_2SO_4) = 0.8 mL.

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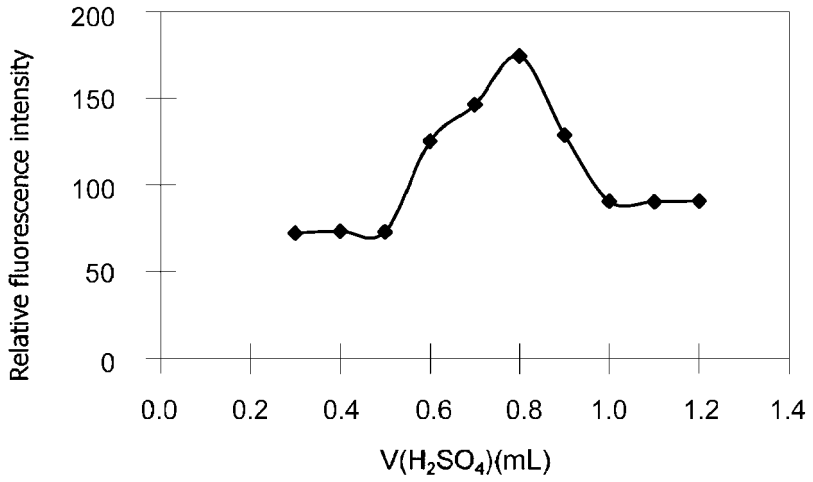

Figure 3. Effect of sulfuric acid concentration on fluorescence of the system. C(Acetamiprid): 4.0×10^{-5} mol L^{-1}; C(ct DNA): 5 μg mL^{-1}.

Effect of the Sulfuric Acid Solution

The concentrations of DNA and acetamiprid were fixed at 5 μg mL^{-1} and 4.0×10^{-4} mol L^{-1}, respectively (Fig. 3). With the change in concentration of sulfuric acid solution, the corresponding change in fluorescence intensity is observed. It can be

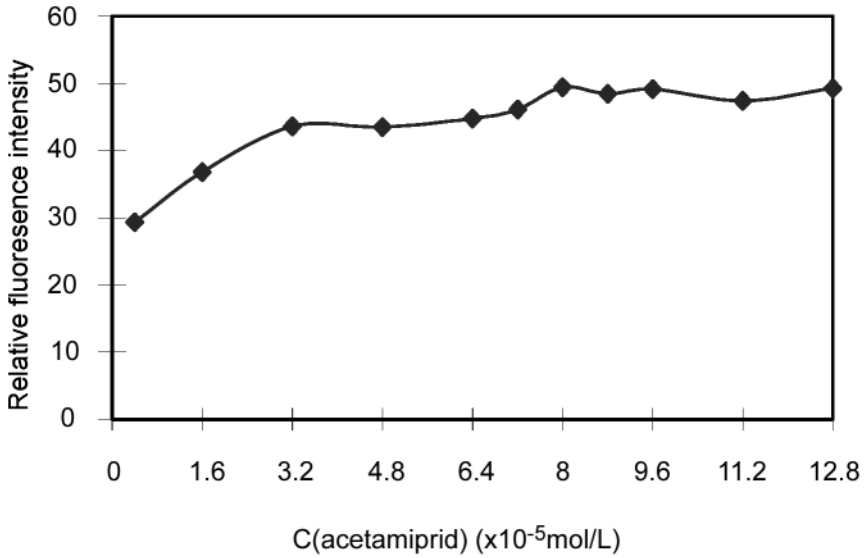

Figure 4. Effect of acetamiprid concentration on fluorescence intensity of the system. C(ct DNA): 4 mg/L; $V(H_2SO_4)$: 0.8 mL.

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seen that the fluorescence intensity of the system is increased with increasing sulfuric acid concentration. When the sulfuric acid concentration is at 1.6×10^{-2} mol L^{-1}, the fluorescence intensity reached maximum. In this paper the concentration of sulfuric acid is chosen as 1.6×10^{-2} mol L^{-1}.

Effect of the Concentration of Acetamiprid

With the change in the concentration of acetamiprid, the intensity difference value ΔF between the system with or without DNA through the change in concentration of acetamiprid were studied. It can be seen that ΔF was highest when the concentration of acetamiprid was 8.0×10^{-5} mol L^{-1}. Therefore the concentration of acetamiprid was chosen at 8.0×10^{-5} mol L^{-1} for further study (Fig. 4).

Effect of Reaction Time

The influence of reaction time on fluorescence intensity was investigated. The results showed that the maximum fluorescence intensity was reached immediately when the solutions were mixed, and remained constant for 45 min. When the incubation time was longer than 45 min, the fluorescence intensity began to decrease. Therefore, a 5 min incubation time was adopted in this work.

The Experiment of Denatured DNA

Double-stranded DNA (dsDNA) was converted into single-stranded (ssDNA) with the opening its double helix by being incubated at 100°C for 15 min and cooled in ice-water bath immediately. The experiments showed that acetamiprid could also react with ssDNA, but the extent of fluorescence enhancement was lower than that by dsDNA. The results support that acetamiprid might strongly interact with dsDNA.

Interference of Other Substance

The influence of some common ions, and bases was studied (see Table 1). It can be seen that $H_2PO_4^-$ and K^+ 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.

Calibration Graph

The calibration graph for the determination was constructed under optimal conditions. The calibration graph is linear over the range of 0.1–10 μg L^{-1}. The limit is 0.06 μg mL^{-1} (S/N = 3). The linear equation was: $\Delta F = 2.72C - 0.25$, and the correlation coefficient r = 0.9996.

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Table 1. Tolerance of foreign substances (determination of $5\,\mu g\,mL^{-1}$).

Substances	Coexisting concentration	Relative error (%)
Adenine	15 mg/L	−3.5
Guanine	10 mg/L	−3.4
$H_2PO_4^-$, Na^+, K^+	5×10^{-5} mol/L	−6.6
Fe^{3+}, Cl^-	1×10^{-7} mol/L	−3.5
Cu^{2+}, SO_4^{2-}	2×10^{-7} mol/L	−3.0
Mn^{2+}, SO_4^{2-}	1×10^{-7}mol/L	−4.5
Cr^{3+}, Cl^-	1×10^{-7} mol/L	−4.2
Ca^{2+}, Cl^-	2×10^{-7} mol/L	−4.9
Zn^{2+}, SO_4^{2-}	2×10^{-7} mol/L	−2.2
Ni^{2+}, SO_4^{2-}	2×10^{-7} mol/L	+3.4
Pb^{2+}, NO^{3-}	1×10^{-7} mol/L	−2.9
Cd^{2+}, SO_4^{2-}	2×10^{-7} mol/L	+3.2
Hg^{2+}, Cl^-	1×10^{-7} mol/L	−5.7
Co^{2+}, Cl^-	2×10^{-7} mol/L	+2.0
Protein BSA	$500\,ng\,mL^{-1}$	−3.5
Protein HSA	$500\,ng\,mL^{-1}$	−4.8

Applying in Sample Determination

The determination of DNA in synthetic samples was conducted with this method. In recovery tests, the standard addition was used. The results are shown in Table 2.

The determination of DNA in yeast was by following procedure. An appropriate amount of yeast solution was transferred to a 10 mL volumetric centrifuge tube. The yeast cells were collected by centrifugation and resuspended in 0.5 mL water. The cells were transferred to a 1.5 mL microfuge tube and collected using a 5s centrifugation. The supernatant was decanted, and a vortex was performed briefly to resuspend the pellet in the residual liquid. The amount of 0.2 mL of 10 mM Tris-HCl buffer solution (pH 8.0) including 2% Triton X-100, 1% SDS, 100 mM NaCl, and 1 mM EDTA was added to the tube; then 0.2 mL phenol-chloroform-isoamyl alcohol (25:24:1) and 0.3 g acid washed glass beads (0.4 mm)

Table 2. Determination result of DNA in synthetic samples.[a]

Sample no.	DNA found ($mg\,L^{-1}$)	DNA added ($mg\,L^{-1}$)	DNA found total ($mg\,L^{-1}$)	Recovery (%)[b]	RSD (%)
1	2.95	2.0	4.87	96.0	3.4
2	2.90	2.0	4.81	95.5	3.6

[a]The composition of the synthetic samples: 1. DNA ($3\,mg\,L^{-1}$); Adenine and guanine ($0.5\,mg\,L^{-1}$); $H_2PO_4^-$ ($1.25 \times 10^{-6}\,mol\,L^{-1}$); Ca^{2+} and Zn^{2+} ($0.5 \times 10^{-8}\,mol\,L^{-1}$) 2. DNA ($3\,mg\,L^{-}$); Adenine and guanine ($1.0\,mg\ L^{-1}$); $H_2PO_4^-$ ($2.5 \times 10^{-6}\,mol\,L^{-1}$); Ca^{2+} and Zn^{2+} ($1.0 \times 10^{-8}\,mol\ L^{-1}$).
[b]Mean of six determinations.

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Table 3. Determination result of DNA in biological samples.

Sample	DNA found ($mg\,L^{-1}$)	DNA added ($mg\,L^{-1}$)	DNA found total ($mg\,L^{-1}$)	Recovery (%)
Yeast	0.102	0.100	0.204	101
Golden staphylococcus	0.240	0.100	0.336	96
		0.200	0.445	103

were added separately. A vortex was performed for 5 min, and then a 0.2 mL TE buffer was added. The tube was spun for 4 min to separate the aqueous layer and the organic layer. The aqueous layer was transferred to a new tube, and 1.0 mL of 100% ethanol was then added. A vortex was performed briefly for mixing. Then, the tube was spun for 2 min to precipitate the DNA pellet. The pellet was resuspended in a 0.4 mL TE buffer. Thirty milligrams of Rnase A was added to the DNA solution, and the solution was incubated at 37°C for 5 min. Ten milliliters of 4M ammonium acetate and 1.0 mL of 100% ethanol were added to the tube. The tube was inverted to mix and then spun for 2 min. The supernatant was decanted, and the pellet was tried. The pellet was resuspended in 200 mL doubly deionized water. Take an appropriate amount of the solution for the determination of DNA by above descript procedure. The determination of DNA in golden staphylococcus sample was also conducted with this method. The original sample of golden staphylococcus was diluted 250-fold for determination. The results are shown in Table 3. It can be seen that the results are satisfactory.

Mechanism of the Reaction Between Acetamiprid and DNA

In Fig. 2, the fluorescence spectra of acetamiprid in presence of DNA were shown. It can be seen that the fluorescence intensity of acetamiprid is significantly enhanced, but the fluorescence maximum at 587 nm remains unchanged. In addition, the fluorescence intensity enhanced by ssDNA is lower than that by dsDNA. The results are in contrast to the observed large red shifts in fluorescent maximum when the fluorescence probe intercalates into the base stack. Therefore, it is suspected that acetamiprid can react with dsDNA and ssDNA in a non-intercalative way. In acidic medium, DNA carry negative electric-charge and protonated acetamiprid carry positive electric-charge. Because they carry different electric-charge, they are attracted to each other. The aggregation of acetamiprid on nucleic acids, as well the presence of hydrogen bond and hydrophobic effect between acetamiprid and DNA, enhance Rayleigh scattering of acetamiprid.

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