Novel Method for the Analysis of Ribonuclease Based on Fluorescence Recovery of a Cationic Aluminum Phthalocyanine-RNA Association Complex as a Red-emitting Fluorogenic Substrate

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The conventional spectrophotometric method that is often applied to determine ribonuclease (RNase) has disadvantages that include cumbersome manipulation, time-consuming processing and a lack of linear range. We had found that a low concentration of RNA could induce cationic aluminum phthalocyanine (tetra(trimethylammonio)aluminum phthalocyanine (TTMAAlPc)), which emitted strong red fluorescence to aggregate in neutral media, resulting in an almost complete quenching of fluorescence from the cationic aluminum phthalocyanine. The RNA is degraded through hydrolysis by RNase, which destroys the induced aggregation of TTMAAlPc on RNA and releases free TTMAAlPc, leading to a significant fluorescence recovery of the reaction system. Based on this new finding, a method to detect RNase by enhanced fluorescence was established using the TTMAAlPc-RNA association complex as a new fluorogenic substrate of RNase. The optimal conditions were determined, and the interfering foreign substances were investigated. Under optimal conditions, the linear range was 0.05 – 50 µg/L, and the detection limit was 0.02 µg/L. This method was applied for the analysis of ribonuclease in urine specimens from normal adults, and the results were consistent with those determined by conventional spectrophotometric methods. The developed method is easy to operate and highly sensitive, and has a wide linear range, thus solving issues with conventional methods. This study applied, for the first time, cationic phthalocyanine as a fluorescent probe in the detection of nuclease, which provides new applications of phthalocyanine as a fluorescent probe emitting at the red wavelength region.

Keywords Ribonuclease, fluorescence, phthalocyanine

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Introduction

Ribonuclease (RNase) is a specific endonuclease for RNA hydrolysis, and it plays an important role in regulating RNA metabolism, gene expression and protein synthesis. In recent years, the relationship of RNase with cancer and other diseases has been a hot topic of research. Studies have found that RNase not only has an anti-tumor effect but also shows anti-proliferation and anti-toxicity functions in cells *in vivo* or *in vitro* through synergistic effects with different types of anti-cancer drugs.¹ The anti-tumor effect of RNase has been confirmed with experiments in cells and nude mice with non-small cell lung cancer and epithelial cancer. RNase is considered to be a promising anti-tumor agent that is expected to replace other anti-cancer drugs, such as cisplatin.^{2–4} Clinically, significant increases in the content of RNase in the serum and urine of patients were reported in studies of the pancreas, renal failure, myocardial infarction, and chronic myelogenous leukemia.^{5,6} A nutritional science study suggested that changes in the level of RNase in urine contributed to the nutritional status of children.⁷ Thus, determining the content of RNase is important.

The conventional methods of RNase detection are mainly ultraviolet (UV) spectrophotometry and gel electrophoresis.⁸ UV spectrophotometry based on the UV absorption change is the most commonly used method because RNA has a characteristic absorption at 260 nm. RNase can hydrolyze the substrate RNA, leading to an increase in the absorbance at 260 nm; the RNase content can then be determined by measuring the change in absorbance before and after the reaction.⁹ Most of the actual measurements of RNase samples reported in the literature, including the Akagi method,¹⁰ Zimmerman method,¹¹ Wagner method¹² and Shapira method,¹³ are based on this principle. However, these methods have cumbersome pre-treatment processes, are time-consuming, and have a limited linear range. Therefore, the establishment of a new method for simple, rapid and sensitive detection of RNase has practical significance.

Because of its excellent photophysical and photochemical properties, phthalocyanine compounds with diamagnetic metal ligands have drawn widespread interest since the 1980s, and research to develop their use as an agent for photochemical diagnoses and treatments remains an important topic in the biomedical field today.^{14,15} In the mid and late 1990s, researchers began investigating the unique fluorescent characteristics of phthalocyanine compounds. Applications of the compound as a fluorescent probe have been considered; however, the researched

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phthalocyanine compounds have mostly been anionic and non-ionic phthalocyanine.^{16,17}

Although the synthesis and application of cationic phthalocyanine compounds have been reported, only a few are available, and they are mostly applicable to photodynamic therapy.¹⁸ Recently, the structure and molecular spectral characteristics of cationic metal phthalocyanine and its interaction with nucleic acid, human serum albumin and surfactants have been reported,¹⁹⁻²¹ but its application as a fluorescent probe for quantitative analysis is still uncommon. The cationic aluminum phthalocyanine (tetra(trimethylammonio) aluminum phthalocyanine (TTMAAlPc)) compound used in this study is a fluorescent probe showing excitation and emission wavelengths in the red wavelength region, and it has a stable fluorescence, high quantum yield, and small emission bandwidth. In this study, we found that anionic RNA could induce the aggregation of TTMAAlPc, resulting in an almost complete quenching of the fluorescence. After adding RNase, the fluorescence of the TTMAAlPc-RNA system was significantly restored. In this study, the TTMAAlPc-RNA association complex serves as a red-emitting fluorogenic substrate for the establishment of a new method of enhanced fluorescence quantitative analysis for RNase, and the reaction mechanism is discussed. This method is simple, fast, sensitive, and specific, and it provides satisfactory results in the detection of actual samples. A literature review has revealed that there are no currently reported studies of cationic phthalocyanine fluorescence compounds for enzyme analysis.

Experimental

Reagents and chemicals

TTMAAlPc was synthesized and purified based on the reported method.²² A stock solution at 1.0×10^{-2} mol/L was prepared. RNA (yeast RNA) was obtained from the Institute of Biochemistry, Chinese Academy of Sciences (Shanghai, China), and RNase A with activity of >60 U/mg was purchased from Sangong Biotech Corp. (Shanghai, China). Other reagents were of analytical grade, and ultrapure water was used.

Apparatus

The following equipment were used in this study: LS-55 fluorescence spectrophotometer (PerkinElmer Inc., USA); electric heated water bath (Shanghai Precision Experimental Equipment Co., China); CHN81801 pH meter (Orion Co., USA); 1 cm quartz cuvette; ultrafiltration tubes (Millipore, USA); electronic analytical balance (Sartorius Corp., Germany); and 5417R tabletop high speed refrigerated centrifuge (Eppendorf Co., Germany).

Experimental method

In a 4-mL centrifuge tube, 300 μ L of phosphate buffer (pH 7.5, 0.2 mol/L), water, 30 μ L TTMAAlPc (1×10^{-4} mol/L), and 11.5 μ L of RNA solution (0.8 g/L) were added with mixing by shaking. The RNase solution or sample for testing was added to a final volume of 3 mL. After mixing well, the reaction was run in a 30°C water bath for 30 min, and then the fluorescence was measured after the reaction system had cooled down to room temperature. The fluorescence excitation and emission wavelengths were set at 610 and 676 nm, with an excitation slit of 5.0 nm and emission slit of 6.0 nm. The tube with no RNA and RNase was the TTMAAlPc tube, and its fluorescence intensity was recorded as I_{TT} . The tube with no RNase solution served as a blank tube, and its fluorescence

Fig. 1 Molecular structure of TTMAAlPc.

intensity was recorded as I_{f0} . The tube containing RNase was the sample tube, and its fluorescence intensity was recorded as I_{f} . The fluorescence quenching factor was represented by $N = I_{\text{fT}}/I_{\text{f0}}$, and the fluorescence recovery factor was represented by $n = I_{\text{f}}/I_{\text{f0}}$.

A slightly modified Akagi ultraviolet spectrophotometry method was used as the control method. Briefly, 1 g of yeast RNA was dissolved in 10 mL of 1 mol/L pH 8.5 phosphate buffer followed by dialysis at 4°C against 0.05 mol/L pH 8.5 phosphate buffer for 72 h, with the dialysate replaced every 24 h. The yeast RNA after dialysis was diluted with 0.05 mol/L pH 8.5 phosphate buffer to a final concentration of 0.5%. The resulting preparation was stored in a 4°C refrigerator for later use. In the measurement, 1 mL of 0.5% yeast RNA was added in a tube, and 50 μ L RNase solution or sample for testing was then added, which was followed by the reaction in a water bath at 37°C for 30 min. To terminate the reaction, 200 μ L of pre-chilled 7.5% perchloric acid (containing 0.05 mol/L nitric acid lanthanum) was immediately added to the tube. For the blank tube, the terminating agent was added before the sample. After an ice bath for 10 min, a centrifugation at 12000 r/min for 5 min was performed using a tabletop centrifuge, and 200 μ L of the supernatant was diluted to 3 mL. The UV absorbance at 260 nm was measured, and the content of RNase in the sample was calculated according to the linear relationship between the absorbance and enzyme concentration.

Results and Discussion

Molecular structure and spectral characteristics of TTMAAlPc

TTMAAlPc (Fig. 1) is a strong fluorescent compound with a porphyrin-like structure. Because a positively charged quaternary ammonium cationic group occurs in each of the four outer periphery benzene rings, its solubility in water is high, which makes it conducive to practical applications. For TTMAAlPc, the fluorescence emission peak (676 nm) is in the red wavelength region, and there are two excitation bands: one is located in the UV region, or the Soret band; and the other is located at the long wavelength region, or the Q band. If the short wavelength is used for excitation, a strong secondary scattering light will occur at its harmonic wavelength (scattering

Fig. 2 Excitation (a) and emission (b) spectra of TTMAAlPc. a, b: $\text{H}_2\text{O} + \text{TTMAAlPc}$, $[\text{TTMAAlPc}] = 1.0 \times 10^{-6} \text{ mol/L}$; Excitation wavelength emission wavelength were set at 610 and 676 nm, respectively.

Fig. 3 Variation in emission spectra of TTMAAlPc with increasing concentrations. The emission spectra of TTMAAlPc were achieved in a phosphate buffer (pH 7.5, 0.2 mol/L). The concentrations of TTMAAlPc from a to g are $5.0 \times 10^{-7} \text{ mol/L}$, $1.0 \times 10^{-6} \text{ mol/L}$, $5.0 \times 10^{-6} \text{ mol/L}$, $1.0 \times 10^{-5} \text{ mol/L}$, $5.0 \times 10^{-5} \text{ mol/L}$, $1.0 \times 10^{-4} \text{ mol/L}$, and $5.0 \times 10^{-4} \text{ mol/L}$, respectively. Excitation and emission wavelengths were set at 610 and 676 nm, respectively.

peak of approximately 700 nm). To avoid the interference from scattering, the excitation wavelength was selected at the Q band region (610 nm).

Figure 2 shows the excitation and emission spectra of TTMAAlPc. In a neutral phosphate buffer, the emission peak of the cationic phthalocyanine was at 676 nm.

Discussion of the reaction mechanism

Fluorescence behavior of the reaction system. TTMAAlPc is a phthalocyanine compound with a diamagnetic central metal ligand that produces a strong red fluorescence. The fluorescence intensity of TTMAAlPc in a dilute solution is enhanced as the concentration of TTMAAlPc increases. When the concentration of TTMAAlPc in the solution reaches a certain level, the TTMAAlPc molecules spontaneously form dimers. Because the dimers usually produce no or weak fluorescence, the fluorescence quenching phenomenon occurs in solutions with a high concentration of phthalocyanine, which is accompanied by a gradual shift of the fluorescence emission peak to the red region. This shift is one of the spectral characteristics of the association reaction of fluorescent phthalocyanine, which is shown in Fig. 3. The experiment showed that the addition of RNA to a

Fig. 4 The fluorescence emission spectra of TTMAAlPc-RNA in the presence of different concentrations of RNase. The emission spectra of TTMAAlPc-RNA were achieved in a phosphate buffer (pH 7.5, 0.2 mol/L). $[\text{TTMAAlPc}] = 1.0 \times 10^{-6} \text{ mol/L}$, $[\text{RNA}] = 3 \text{ mg/L}$. The concentrations of RNase added for curves 1 - 7 were: 0, 1.0, 2.5, 5.0, 10, 25 and 50 $\mu\text{g/L}$, respectively. Excitation and emission wavelengths were set at 610 and 676 nm, respectively. The photos at the upper right corner were taken in the media as follows: A. TTMAAlPc + RNA + phosphate buffer (pH 7.5, 0.2 mol/L), $[\text{TTMAAlPc}] = 1.0 \times 10^{-6} \text{ mol/L}$, $[\text{RNA}] = 3 \text{ mg/L}$; B. TTMAAlPc + RNA + RNase + buffer, $[\text{TTMAAlPc}] = 1.0 \times 10^{-6} \text{ mol/L}$, $[\text{RNA}] = 3 \text{ mg/L}$, $[\text{RNase}] = 90 \mu\text{g/L}$. A portable UV lamp was used for irradiation.

Fig. 5 Variations in the fluorescence intensity of TTMAAlPc with increasing concentrations of RNA. The plot was constructed based on the fluorescence intensity *versus* increasing concentrations of RNA in a phosphate buffer (pH 7.5, 0.2 mol/L). $[\text{TTMAAlPc}] = 1.0 \times 10^{-6} \text{ mol/L}$. The concentrations of RNA for curves varied from 0.5 to 400 mg/L. Excitation and emission wavelengths were set at 610 and 676 nm, respectively.

dilute TTMAAlPc solution caused significant fluorescence quenching with almost no fluorescence in an appropriate quality ratio of TTMAAlPc/RNA (0.22). This result occurred because the phosphate groups carried by RNA were exposed on the surface of the molecule, producing negative charges for the entire RNA molecule to induce aggregation of positively charged TTMAAlPc on the RNA to form the association complex, which led to the fluorescence quenching of the system.

Fig. 6 Influence of reaction time on the fluorescence anisotropy of the reaction system. The plot was achieved based on the fluorescence anisotropy *versus* reaction time of the solution consisting of TTMAAIpc, RNA, RNase and a phosphate buffer (pH 7.5, 0.2 mol/L). [TTMAAIpc] = 1.0×10^{-6} mol/L, [RNA] = 200 μ g/mL and [RNase] = 10 μ g/L. Excitation and emission wavelengths were set at 610 and 676 nm, respectively.

When RNase was added to the TTMAAIpc-RNA association system, the RNA was hydrolyzed to the oligonucleotide and the TTMAAIpc that was gathered around the RNA was gradually released, which gradually restored the fluorescence (Fig. 4). The method established in this study was based on this principle.

Fixing the concentration of TTMAAIpc and changing the concentration of RNA, an I_0 -c curve (Fig. 5), which was also called titration curve of RNA on the TTMAAIpc, was achieved. The titration curve shows that fluorescence quenching recovered to a stable value after reaching the lowest point, and the corresponding RNA concentration achieved full association with TTMAAIpc (200 μ g/mL). The presence of RNA in a low concentration strongly induced an association with TTMAAIpc and resulted in significant fluorescence quenching in the system. The continued addition of RNA would result in the gradual dimerization of TTMAAIpc as the RNA concentration increased; therefore, the fluorescence recovered and eventually reached a stable value.

Fluorescence anisotropy of the reaction system. To further explore the reaction mechanism, the hydrolysis reaction was investigated using the steady-state fluorescence anisotropy technique. Fluorescence anisotropy (commonly referred to in the literature as fluorescence polarization) is widely applied to investigate interactions between bio-macromolecules and small molecules.^{23,24} The small molecule fluorescent probe moves rapidly because of its small molecular weight, and its fluorescence emission is nearly depolarized. After interacting with a macromolecule, the small molecule fluorescent probe is significantly increased in fluorescence anisotropy. Thus, fluorescence anisotropy measurements can provide information on the interactions between small molecule and biological macromolecules.²⁵

After interacting with RNA, TTMAAIpc showed a decreased rate of movement and its fluorescence anisotropy (expressed as "r") increased. The titer of the full association concentration was obtained by RNA on the TTMAAIpc titration curve (200 μ g/mL). When TTMAAIpc and RNA were fully associated, RNase was added to detect changes of fluorescence anisotropy in the system over time, and it resulted in the r-t

Fig. 7 Fluorescence quenching of RNA on TTMAAIpc under different quality ratios of [TTMAAIpc]/[RNA]. [TTMAAIpc] = 1.0×10^{-6} mol/L, quality ratios of [TTMAAIpc]/[RNA] were from 0.08 to 0.28, and a phosphate buffer (pH 7.5, 0.2 mol/L) was used. Excitation and emission wavelengths were set at 610 and 676 nm, respectively.

curve (a curve of fluorescence anisotropy over temperature, Fig. 6). Accordingly, as the reaction time is extended, the fluorescence anisotropy in the system decreased. This phenomenon is consistent with expectations because the RNA in the TTMAAIpc-RNA associated complex was hydrolyzed to oligonucleotide fragments, which led to an increase in the moving rate of TTMAAIpc and to a decrease in fluorescence anisotropy. This result indicates that the substrate RNA labeled with TTMAAIpc was gradually hydrolyzed by RNase, which further confirms the previously described speculations on the reaction mechanism.

Optimization of the experimental conditions

Optimization of the TTMAAIpc/RNA ratio. The effect of the TTMAAIpc/RNA ratio on fluorescence quenching is shown in Fig. 7. The TTMAAIpc/RNA ratio of mass showing the highest fluorescence quenching factor for TTMAAIpc was 0.22, which indicates that the TTMAAIpc concentration in the system was 1×10^{-6} mol/L and that the RNA concentration was 3 mg/L.

Optimization of the buffer system and pH. According to the literature, the optimal pH for RNase is 7.0–7.5. The impact of the commonly used buffer systems in the pH range of 6.0–8.0, including phosphate buffer, Tris-HCl buffer, Britton-Robinson buffer, and extensive buffer (prepared by mixing citric acid, dipotassium hydrogen phosphate, boric acid, barbitone and sodium hydroxide), on the change in fluorescence intensity was investigated. As shown in Fig. 8, the fluorescence recovery factor ($n = I_f/I_0$) was highest in the pH 7.5 phosphate buffer.

Selection of the reaction time. The reaction system at room temperature reached equilibrium after 30 min, and the fluorescence intensity remained stable within 24 h, indicating good stability of the system. Therefore, the reaction time of 30 min was selected in this study.

Selection of the reaction temperature. The effect of different reaction temperatures on the reaction system was investigated (Fig. 9). In the range of 20–90°C, the fluorescence recovery factor initially increased with increasing temperature, with the fluorescence recovery factor of the system achieving maximum values 30°C. When the temperature was above 30°C, the fluorescence recovery factor decreased, and when the

Fig. 8 Fluorescence recovery based on different pH values and different buffers. The plot was achieved based on the fluorescence variation *versus* different pH values. [TTMAAlPc] = 1.0×10^{-6} mol/L, [RNA] = 3 μ g/mL and [RNase] = 10 μ g/L. Excitation and emission wavelengths were set at 610 and 676 nm, respectively. The buffer media for curves a to d were : a. phosphate buffer (0.2 mol/L, pH 6.0 – 8.0); b. extensive buffer (this buffer makes up of 0.2 mol/L citric acid, potassium hydrogen phosphate, boric acid, barbitone and sodium hydroxide, pH 6.0 – 10.0); c. Britton-Robinson buffer (0.2 mol/L, pH 6.0 – 10.0); d. Tris-HCl buffer (0.05 mol/L, pH 7.0 – 9.0).

Fig. 9 The influence of temperature on the degree of fluorescence recovery. The reaction system composed of TTMAAlPc, RNA, RNase and a phosphate buffer (pH 7.5, 0.2 mol/L). [TTMAAlPc] = 1.0×10^{-6} mol/L, [RNA] = 3 μ g/mL and [RNase] = 10 μ g/L. Excitation and emission wavelengths were set at 610 and 676 nm, respectively.

temperature was higher than 60°C, the fluorescence of the system declined rapidly. Therefore, 30°C was selected for the reaction.

Impact of TTMAAlPc on RNase activity

To verify whether the detected RNase activity is affected by cationic aluminum phthalocyanine, the impact of TTMAAlPc on RNase activity was investigated by the Akagi UV spectrophotometry method as previously described. The TTMAAlPc test group consisted of TTMAAlPc added to both the sample and blank substrate RNA to obtain a final TTMAAlPc concentration of 1×10^{-6} mol/L, which was followed by the

Table 1 Impact of TTMAAlPc on RNase activity

	RNase activity (A)						$\bar{x}$	RSD, %
	1	2	3	4	5	6		
Test group	1.420	1.511	1.485	1.513	1.509	1.439	1.479	2.73
Control group	1.497	1.409	1.490	1.509	1.487	1.447	1.473	2.56

Fig. 10 The calibration curve of RNase. Excitation and emission wavelengths for the construction of the calibration curve were set at 610 and 676 nm, respectively. [TTMAAlPc] = 1.0×10^{-6} mol/L and [RNA] = 3 μ g/mL, A pH 7.5 phosphate buffer (0.2 mol/L) was used in the determination. The standard deviation for the calibration curve is 0.67 – 3.74%, which is within the permissible error (5%).

procedure described for the control group without TTMAAlPc. Table 1 showed that the average absorbance of the TTMAAlPc test group (A) was 1.48, which was basically the same as the control group without TTMAAlPc (1.47, $n = 6$), indicating that there was no influence of TTMAAlPc on RNase activity.

Plot of the calibration curve

Under optimal conditions, the fluorescence intensity of the system at different concentrations of RNase was measured, and the logarithm of the fluorescence intensity showed a good linear relationship with the logarithm of the RNase concentration in the range of 0.050 – 50.0 μ g/L. Based on the curve of the logarithm of RNase concentrations (C, mg/mL), which is $\log(C)$ of the logarithm of the recovery of fluorescence intensity (I_t) $\log(I_t)$, a linear regression equation could be obtained: $y = 0.37x + 3.43$ ($r = 0.9974$), and it had a linear range of 0.050 – 50.0 μ g/L and a detection limit of 20 ng/L (Fig. 10).

Impact of the coexisting substances

The influence of common metal ions, amino acids, proteins, and other interfering substances in the system on the detection of RNase was investigated (Table 2). The RNase concentration for the test was 30.0 μ g/L, and the maximum permissible error was 5%. The results showed that the presence of general substances did not affect measurements in the system, indicating that this method has a strong anti-interference ability with ions and small molecules. However, biological macromolecules, such as DNA and proteins, in the system showed a large degree of interference, which may have resulted from the involvement of other biological macromolecules during competitive binding

Table 2 Influence of coexisting substances on the detection of RNase (RNase: 30 µg/L)

Coexisting substance	Concentration/mol L ⁻¹	Relative error, %	Coexisting substance	Concentration/g L ⁻¹	Relative error, %
Ba ²⁺	5.00 × 10 ⁻⁴	+2.22	Leucine	1.50 × 10 ⁻³	+3.30
Ca ²⁺	1.00 × 10 ⁻⁴	-2.25	Threonine	1.50 × 10 ⁻³	+1.30
Cu ²⁺	1.00 × 10 ⁻⁴	-2.64	Proline	1.50 × 10 ⁻³	-1.87
Mn ²⁺	1.00 × 10 ⁻⁴	-0.88	Methionine	1.50 × 10 ⁻³	+2.17
Zn ²⁺	1.00 × 10 ⁻⁵	-4.52	Histidine	1.50 × 10 ⁻³	-1.27
Al ³⁺	1.00 × 10 ⁻⁴	-2.94	Boric acid	1.00 × 10 ⁻²	-3.42
F ⁻	5.00 × 10 ⁻⁵	+3.52	SDS	2.72 × 10 ⁻²	-3.89
Br ⁻	5.00 × 10 ⁻⁴	-3.73	Triton X-100	1.94 × 10 ⁻¹	-3.63
I ⁻	1.00 × 10 ⁻⁴	-3.63	Tween 80	4.30 × 10 ⁻²	-4.33
NO ₃ ⁻	5.00 × 10 ⁻⁴	-1.52	Glycerin	9.20 × 10 ⁻³	+1.53
EDTA	3.00 × 10 ⁻⁵	-6.16	Sucrose	1.00 × 10 ⁻²	-3.49
CTAB	1.00 × 10 ⁻⁵	+6.16	Vitamin B6	1.00 × 10 ⁻³	+2.49
CTAC	1.00 × 10 ⁻⁵	+3.18	Herring sperm DNA	3.00 × 10 ⁻⁶	-3.36
Ethanol	1.00 × 10 ⁻⁴	-1.73	Salmon sperm DNA	1.00 × 10 ⁻⁶	-3.06
			Calf thymus DNA	1.00 × 10 ⁻⁶	-4.19
			Bovine serum albumin	2.00 × 10 ⁻⁶	-4.20
			Human serum albumin	1.50 × 10 ⁻⁶	-4.39

The standard deviation for the calibration curve is about 0.55 – 3.08%; control within the maximum permissible error of 5%.

Table 3 Analytical results of RNase in normal urine samples

Sample	Content of RNase		Relative standard deviation (<i>n</i> = 7), %
	Akagi method as control/µg mL ⁻¹	Experimental method/µg mL ⁻¹	
1	0.287	0.277	3.14
2	0.431	0.453	4.91
3	0.434	0.408	0.90

to TTMAAIPc.

Detection of real samples

The method established in this study was used to detect the RNase content in actual urine specimens, and the results were compared with the conventional Akagi UV spectrophotometry method. The two methods were consistent with each other.

Normal adult urine (200 µL) was added to an ultrafiltration centrifuge tube and centrifuged at 12000 r/min for 8 min. The remaining liquid in the centrifuge tube was collected and served as the samples for testing. The samples were measured as described in the methods section, and the calibration curve for RNase was plotted simultaneously. The content of RNase in the sample was calculated. The control method (Akagi UV spectrophotometry) was conducted as described in the methods section, and the results are shown in Table 3.

Conclusions

Based on the principle of ion association, this study used the TTMAAIPc-RNA association complex as a red-emitting fluorogenic substrate for RNase to establish a new method of RNase quantitative analysis, the mechanism was explored using steady-state fluorescence spectra and the fluorescence anisotropy technique. Compared with conventional methods, this method is more simple and rapid and has a wider linear range, thus achieving a simple and rapid method of determining the RNase in actual samples. This study present new applications of cationic phthalocyanine, a red-emitting fluorescent probe, in

enzyme assay.

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