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SHORT COMMUNICATION

SPECTROPHOTOMETRIC DETERMINATION OF YEAST RNA WITH NEUTRAL RED

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ABSTRACT. The interaction of neutral red (NR) with yeast RNA (yRNA) was studied by UV-Vis spectrophotometry to develop a simple spectrophotometric method for the determination yRNA. NR exhibited a maximum absorption peak at 528 nm in a Britton-Robinson (B-R) buffer solution of pH 4.0. After the addition of yRNA into NR solution, the absorbance value was greatly decreased and no new absorption peaks appeared. The interaction conditions such as the buffer pH, reaction time, etc. were carefully studied. Under the optimal conditions the decrease in absorbance value was proportional to the yRNA concentration in the range from 0.2 to 20.0 mg L^{-1} when 8.0×10^{-5} M NR was employed. The detection limit was calculated as 0.78 mg L^{-1} (3σ) and three synthetic samples were determined satisfactorily. A binding ratio of NR to yRNA was found to be 1:1 by the molar ratio method.

KEY WORDS: Neutral red, Yeast RNA, Interaction, UV-Vis spectrophotometry

INTRODUCTION

The determination of the content of nucleic acids (NAs) is very important in mutation detection and clinical diagnostics for its specific functions in biochemistry. Many methods had been proposed for the NAs determination such as spectrophotometry [1, 2], fluorescence [3], light-scattering technique [4] and electrochemical methods [5]. However most of them are concerned with deoxyribonucleic acid (DNA). To our knowledge there are few reports on the determination of ribonucleic acid (RNA). Evidence indicates that proteins may take advantage of the conformational polymorphism in the RNA backbone in recognizing specific binding sites on the macromolecule. So it is necessary to establish a sensitive method to detect RNA. Some transition metal chelates such as rhodium(III) phenanthroline, ruthenium(II) polypyridine, zinc(II) imidazole, etc. have served as the selective probes for RNA recognition and hydrolysis, which could be used to better understand the structure and nature of RNA [6-9]. Recently, Sun *et al.* investigated the interaction of pyronine B with RNA by an electrochemical method [10]. Si discussed the mechanism of RNA interaction with neutral red by spectrophotometry and drawn a conclusion that an electrostatic binding resulted in the interaction [11]. Spectrophotometry is a simple method and has been used for the determination of biomolecules such as proteins and glycosaminoglycans [12, 13]. In this study, neutral red (NR) was selected as a spectrophotometric probe to establish a method for yeast RNA (yRNA) determination. The structure of NR is shown in Figure 1. In a pH 4.0 Britton-Robinson (B-R) buffer solution, the interaction of yRNA with NR resulted in the changes of UV-Vis absorption spectrum, which could be further used for yRNA detection. Under the optimal conditions, the binding ratio was determined spectrophotometrically.

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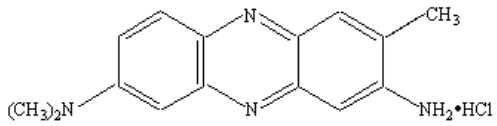

Figure 1. The molecular structure of neutral red.

EXPERIMENTAL

Apparatus and reagents. A Cary 50 probe spectrophotometer (Varian, Australia) was used for recording absorption spectra and a 721 E UV-Vis spectrophotometer (Shanghai Spectra Instrumental Company, China) was used to measure the absorbance at a fixed wavelength using a 1-cm path length. The pH measurements were made with a pH-25 acidimeter (Shanghai Leici Instrument Factory, China). All the experiments were carried out at 25 ± 2 ºC.

Yeast ribonucleic acid (yRNA, Tianjin Damao Chemical Reagents Company, China) was used as received without further purification. A 1.0 g L^{-1} stock solution of yRNA was prepared by dissolving 0.1000 g of yRNA in doubly distilled water, diluted to 100 mL and stored at 4 ºC. The working solutions were obtained by diluting the stock solution with water. The concentration of yRNA was determined by measuring the absorbance at 260 nm (ε_{RNA}= 7800 L mol^{-1} cm^{-1}). A 1.0 × 10^{-3} M neutral red (NR, Shanghai 3rd Chemical Reagent Factory, China) solution was prepared by dissolving 0.02880 g NR into water and diluted to 100 mL. 0.2 M pH 4.0 Britton-Robinson (B-R) buffer solution was used to control the acidity of the interaction solution. All the chemicals used were of analytical reagents grade and double-distilled water was used throughout.

Procedure. 0.5 mL of 1.0 × 10^{-3} M NR solution, 2.0 mL of pH 4.0 B-R buffer solution and an appropriate amount of standard yRNA solution were added in that order into a 10 mL calibrated tube. The mixture was diluted to the mark with water and shaken homogeneously, then allowed to stand at 25 °C for 20 min. The absorbance of the mixed solution (A) was measured with the B-R solution as reference. Under the same conditions the absorbance of the blank solution (A_0) without the addition of yRNA was recorded and the difference in absorbance ($\Delta A = A_0$-A) was used to determine yRNA.

RESULTS AND DISCUSSION

Characteristics of UV-Vis absorption spectra. The UV-Vis absorption spectra of NR in the absence and presence of yRNA are shown in Figure 2. In pH 4.0 B-R buffer solution, NR had a maximum absorption band at 590 nm (curve 1). When yRNA solution was added, a significant hypochromic effect was observed and the absorbance decreased (curve 2). The results indicated that NR and yRNA had interacted in the mixture solution. Based on the decrease of absorbance value, a simple and sensitive spectrophotometric method was established for yRNA determination.

Optimization of general procedures. The influence of acidity on the binding reaction was investigated because the pH of solution could influence the electrical properties of NR and the natural structure of yRNA. In the pH range from 3.0 to 8.0, the value of ΔA reached its maximum at pH 4.0, so this pH was selected for the assay. When the pH is too high or too low, the yRNA may be denatured. The amount of 0.2 M B-R buffer solution was selected with the results as 0.5 mL of B-R buffer used in a final 10 mL solution.

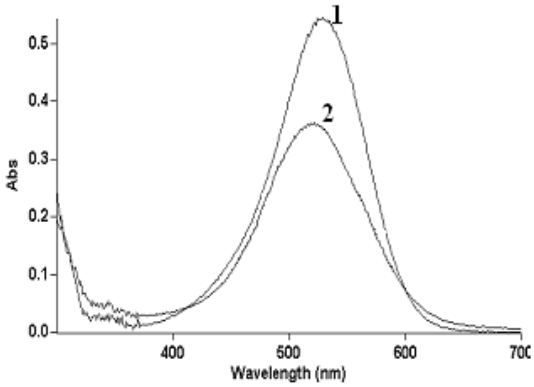

Figure 2. Absorption spectra of NR-yRNA reaction system. Condition: $1.5.0 \times 10^{-5}$ mol L^{-1} neutral red; 2.1 + 50.0 mg L^{-1} RNA.

The effect of the concentration of NR on the binding reaction was studied by fixing yRNA concentration at 10.0 mg L^{-1}. When the concentration of NR was at 8.0×10^{-5} M, the difference of absorbance value reached its maximum, so the final concentration of NR was selected as 8.0×10^{-5} M in this experiment.

The adding sequence of buffer, NR and yRNA was investigated and the results showed that the best addition sequence was NR, B-R buffer and yRNA. The results indicated that the electronic coupling made NR bind to yRNA.

The influence of ionic strength on the reaction of NR and yRNA was also carefully studied by adding different amount of NaCl in the mixture. The results showed that the absorbance did not change significantly in the NaCl concentration range of 10^{-3} to 0.1 M. When the concentration of NaCl was more than 0.1 M, the value of ΔA decreased greatly, which indicated that the interaction was caused by electrostatic attraction.

The influence of the reaction time was investigated at the temperature of 25 ºC and the results indicated that the binding reaction reached the equilibrium for about 20 min. The absorbance value remained constant for about 1 hour and the reaction solution showed good stability.

Tolerance of foreign substances. The influences of coexisting substances such as metal ions, amino acids and glucose on the determination of 10.0 mg L^{-1} yRNA were tested and the results are given in Table 1. The results indicated that most of these substances did not interfere with the determination and this analytical method had good selectivity.

Table 1. Tolerance of coexisting substances on the determination of 10.0 mg L^{-1} yRNA.

Coexisting substances	Concentration (M)	Relative error (%)	Coexisting substances	Concentration (mg L^{-1})	Relative error (%)
Ca^{2+}	5.0×10^{-5}	1.86	Citric acid	5.0	0.41
Fe^{3+}	5.0×10^{-5}	2.51	Glycine	5.0	1.64
Cu^{2+}	5.0×10^{-5}	-2.43	L-Tyrosine	5.0	-2.34
Co^{2+}	5.0×10^{-5}	-3.71	L-Leucine	5.0	-1.03
Sn^{2+}	5.0×10^{-5}	0.41	L-Cysteine	5.0	-3.08
Mn^{2+}	5.0×10^{-5}	6.19	L-Glutamine	5.0	1.23
Mg^{2+}	5.0×10^{-5}	1.65	L-Arginine	5.0	-2.53
Zn^{2+}	5.0×10^{-5}	-10.10	L-Valine	5.0	-4.93
Glucose	5.0 (mg L^{-1})	5.88	L-Serine	5.0	3.70

Calibration curve. The relationship of ΔA against the yRNA concentration was established under the optimal conditions. The linear regression equation obtained was ΔA = 0.017 C (mg/L)-0.0567 (n = 9, γ = 0.997) with the yRNA concentration range from 0.2 to 20.0 mg L^{-1}. The relative standard deviation (RSD) of 11 parallel determinations of 10.0 mg L^{-1} yRNA was 2.74 % with the detection limit of yRNA estimated as 0.78 mg L^{-1} (3σ).

Sample determination. Three synthetic samples containing amino acid, metal ions and yRNA were determined using the proposed method and the results are given in Table 2. It can be seen that the results are satisfactory with the recovery in the range from 99.4 % to 102.7 %.

Table 2. Determination results of yRNA in synthetic samples (n = 5).

Samples	Coexisting substances	Added (mg L^{-1})	Found (mg L^{-1})	RSD (%)	Recovery (%)
1	L-Serine, glycine, Zn^{2+}, Co^{2+}	5.00	4.97	-0.6	99.4
2	L-Leucine, glycine, Mg^{2+}, Sn^{2+}	10.00	10.27	2.70	102.7
3	Glycine, L-serine, Mg^{2+}, Co^{2+}	15.00	15.04	0.27	100.3

*Concentration of coexisting substances: L-serine, glycine, L-leucine: 0.01 mg L^{-1}; Sn^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}: 1.0×10^{-5} M.

Measurement of the binding ratio. The binding ratio of NR with yRNA was determined by the commonly used molar ratio method. The results obtained showed the binding ratio of NR-yRNA complex obtained was about 1.

CONCLUSIONS

The interaction of NR with yRNA was studied by UV-Vis spectrophotometric method. Under the optimal conditions, a simple and sensitive spectrophotometric method for yRNA detection was established and successfully applied to the synthetic samples determination.

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