A NEW CHEMILUMINESCENCE REACTION OF NUCLEIC ACID AND ITS ANALYTICAL APPLICATION

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Abstract. A new chemiluminescence reaction of ribonucleic acid (RNA) has been studied based on the RNA reacting with hydrogen peroxide and ascorbic acid in the presence of copper (II) and 1,10-phenanthroline (phen) in pH5.5 medium. Under optimal reaction condition, a new chemiluminescence (CL) method for the determination of RNA was developed. RNA in the range 0-4 μ g/ml and 5-18 μ g/ml has been determined with good precision. The detection limit for RNA was 0.08 μ g/ml.

Key words nucleic acid, chemiluminescence reaction.

It is well known that nucleic acid probes are of great importance in molecular biology and clinical diagnostics. Many methods (e.g. to label DNA/ or RNA with radioisotopes, enzymes and luminescent reagents) have been reported. Recently, in our laboratory, we discovered a new chemiluminescence reaction of nucleic acids (RNA and DNA) with hydrogen peroxide and ascorbic acid (VC) in the presence of copper(II) and 1,10-phenanthroline(phen). In this paper we report the new chemiluminescence reaction of yeast ribonucleic acid.

EXPERIMENTAL

Apparatus The relative intensity of chemiluminescence(I_{CL}) was measured with an LKB-Wallac 1251 luminometer.

Reagents Yeast ribonucleic acid(RNA) was obtained from Shanghai Institute of Biochemistry, and used without further purification. Other reagents were of analytical reagent grade. The stock solution of RNA(100 μ g/ml) was prepared by dissolving appropriate amount of RNA in 0.05M sodium perchlorate solution with deionized-distilled water.

Procedure To a 10 ml volumetric tube, added 0.5 ml of 2×10^{-3} M solution of cupric sulphate, 0.5ml of 1×10^{-2} M solution of phen, 2.0ml of 0.1M acetic buffer solution (pH5.5) and an aliquot solution of RNA(containing 0-100 μ g RNA), diluted to 10ml with water and mixed well. After that, transferred 500 μ l of the obtained solution to a measurement tube in LKB-Wallac 1251 luminometer and incubated it at 37°C for 15 min. Then ascorbic acid and H₂O₂ were added into the tube simultaneously and recorded the CL intensity.

RESULTS AND DISCUSSION

The kinetic curve of chemiluminescence reaction of RNA was shown in Fig.1. The blank exhibited weak luminescence. There were great difference in time for reaching maximum intensity of CL(t_{max}) between the blank and the RNA. At the time when RNA reached emission maximum, the CL signal from the blank almost decayed to a very low, constant level.

The optimum condition for the RNA chemiluminescence reaction is: pH5.2-5.6, [Cu²⁺]= 1×10^{-4} M, [phen]= 5×10^{-4} M, [VC]= 6.6×10^{-4} M - 11.6×10^{-4} M, [H₂O₂] $\geq 1.6\%$ (V/V). In our study, acetic buffer solution(pH5.5) was chosen because it could provide higher and repeatable chemiluminescence. Hydrogen

peroxide(2.0%) and VC($6.6 \times 10^{-4} \text{M}$) must be added simultaneously, it could give highest intensity of CL.

Fig.1. Kinetic curve of CL of RNA

1. phen/ Cu^{2+} / H_2O_2 /VC
2. RNA/phen/ Cu^{2+} / H_2O_2 /VC
phen: $5 \times 10^{-4} \text{M}$; Cu^{2+} : $1 \times 10^{-4} \text{M}$;
 H_2O_2 : 2.0%(V/V); VC: $6.6 \times 10^{-4} \text{M}$;
pH 5.5.

In the temperature range ($25^\circ\text{C} - 41^\circ\text{C}$) tested, the CL intensity increased steadily and the time for peaking out in CL($t_{\max}$) decreased sharply. We selected 37°C as the reaction temperature.

On the basis of above study, a new chemiluminescence method for the determination of RNA has been developed. RNA in the range 0 - $4 \mu\text{g/ml}$ and 5-18 $\mu\text{g/ml}$ has been determined with precision (RSD was 3.9% for 0.2 $\mu\text{g RNA/ml}$). The detection limit for RNA was 0.08 $\mu\text{g/ml}$.

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