DNA Chemiluminescence by Phenanthroline-Cu(II)

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Introduction

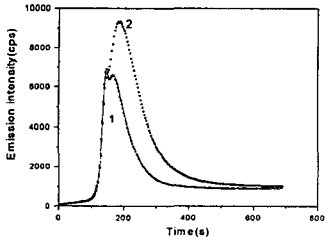
It is belived that phenanthroline-Cu(II) complex has nuclease activity. Its damage to DNA is attributed to the reactive oxygen species (ROS) generated [1,2]. Although the chemical nature of these oxidative lesions has been attentively studied, there is still much uncertain on the mechanism. Recent finding that DNA damage induced by Phen-Cu(II)/ascorbate/H_2O_2 was accompanied by light emission led to initiate the studies on chemiluminescent character of this system in order to investigate the mechanism of interaction between the phenanthroline-Cu(II) complex and DNA and some potential application as a technique such as its potential use in antioxidation evaluation [3].

Materials and methods

Chemiluminescence of DNA, bases and nucleotides. Copper and 1,10-phenanthroline was premixed in 0.1M NaOAc/HOAc (pH 5.2) buffer, a moderate concentration of DNA or different bases and nucleotides was incubated with Cu-Phen at 18°C for 5 minutes. Following this ascorbate and H_2O_2 were added without interval to the solution to give final volume 1.2 ml. The kinetic curve of chemiluminescence produced in the phen-Cu(II)/H_2O_2/ascorbate system was immediately recorded with a type BJL Ultra-Weak Chemiluminescence system (Jye Horn Business Co. LTD), The voltage in the photomultiplier was kept at 875V.

Results and Discussion

1. DNA chemiluminescence and its emission spectra



The kinetic CL curve contains two peaks (A and B) after adding the hydrogen peroxide to the complete system containing phenanthroline-Cu(II), DNA and ascorbate in phosphate buffer(Fig.1-A, curve 2). two emission peaks were observed with maximal emissions at 400-420 (A) and 460-480(B) respectively. The peak A has been assigned to the self-oxidation of phenanthroline[4]. For the peak B, a delayed and much stronger, its emission intensity increases linearly with increasing DNA concentration. Addition of H_2O_2 alone, Cu2+ alone or ascorbate alone show no chemiluminescence, only when Cu2+-Phen and H2O2 are both in solution, DNA Chemiluminescence can be observed.

2. The base specificity of chemiluminescence

Compared at the same concentrations of the five common bases(A,G,C,T,U), only guanine can give rise to chemiluminescence which was similar to DNA. The different substitutes on guanine residue markedly influrence the emission intensity of CL The overall order is: G<GR<dGR <GMP <GTP <dGMP<dGTP; Addition of O^6mdGMP or N^7mdGMP produce little Chemiluminescence (Table.1). It suggests that any factors that can influence the oxidizability of guanine will lead to changing in its CL property.