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Electrochemical cleavage of DNA in the presence of copper–sulfosalicylic acid complex

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Abstract

Electrochemical cleavage of DNA in the presence of copper–sulfosalicylic acid [$Cu(ssal)_2^{2+}$] complex was studied. The cleavage was observed in a certain potential region where redox cycling of $Cu(ssal)_2^{2+}/Cu(ssal)_2^{+}$ took place. $Cu(ssal)_2^{2+}$ complex mediate generation of reactive oxygen species from O_2 by the Fenton reaction, these radicals are capable of damaging DNA. The cleaved DNA fragments were separated by high-performance liquid chromatography (HPLC). The experimental results indicated that the method for electrochemical cleavage of DNA by $Cu(ssal)_2^{2+}$ complex was simple and efficient.

Keywords: Electrochemical; DNA cleavage; $Cu(ssal)_2^{2+}$ complex; HPLC

1. Introduction

DNA cleavage induced by oxygen radicals has been implicated to be important in ageing, mutagenesis and carcinogenesis [1–4]. Some metal ions and complexes can undergo one-electron redox reactions (such as copper, iron or manganese) producing reactive oxygen species, the latter then damage DNA, frequently yielding strand breaks [5]. Copper is recognized as an essential metal element widely distributed in the biological system such as cells and body. It is a bioessential element with relevant oxidation states +1 and +2. Coordination compounds of copper have been extensively used in metal-mediated DNA cleavage [6–11].

There are several strategies to induce redox reaction of copper ion. Copper ion can be reduced in the presence of reducing agents such as thiols, ascorbate, NADH or phenol compounds, the reduced copper ion react with hydrogen peroxide producing hydroxyl radicals in a one-electron redox reaction. These hydroxyl radicals attack the DNA double strands to cause strands cleavage. However, extra reductant and H_2O_2 are required in the reaction system. It caused that the conditions of DNA cleavage reaction were obviously different from physiological conditions. Since copper ion can react with oxygen producing reactive oxygen in redox reactions, using electrochemical methods to cleave DNA has reached much attention. Fojta et al. [12] studied DNA cleavage by reactive oxygen species generated via a modulated electrochemically reaction of copper ions on a hanging mercury drop electrode in the presence of hydrogen peroxide and/or oxygen. According to height of DNA peak 3 (E_p=−1.43 V) increasing, the cleavage of DNA was ascertained. Labuda et al. [13] studied DNA cleavage using a DNA modified glassy carbon electrode in the presence of a copper-1,10-phenanthroline complex by examining oxidative current of base, the break of DNA strands was observed. Rodriguez et al. [14] studied nicking of supercoiled (sc) DNA by oxygen reduction at electrode in the presence of transition metals, observing potential-modulated DNA cleavage during electrolysis of aerobic DNA solution. In these studies, all the reduced metal ions or complexes reacted with oxygen in solution and active oxygen species were certainly generated bring about the DNA strand break

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and its system of DNA cleavage reaction is simple and the oxidation state of the redox-active species at electrode surface can be modulated.

Here, we chose electrochemical method to induce DNA cleavage in presence of $Cu(ssal)_2^{2+}$ complex. The influence of several experimental conditions including electrode potential, electrolysis time and the concentration of $Cu(ssal)_2^{2+}$ complex on DNA cleavage was studied in detail. The experimental results revealed that the proposed method for cleaving DNA is highly efficient.

2. Experimental section

2.1. Apparatus and regents

CHI660A electrochemical analyzer (Shanghai Chenhua Apparatus, China) and Model 363 potentiostat/galvanostat (EG&G Pinceton Applied Research) were applied. L-7100 high performance liquid chromatography (Hitachi High Technologies, Tokyo, Japan) equipped with L-7420 UV–Vis spectrophotometric detector. The Shim-pack Diol-300 chromatographic column (7.9 mm∅×25 cm) packed with 5-μm porous silica microsphere (pore diameter 300 Å), which have a diol-bonded phase, was employed to separate the cleaved DNA fragments. Calf thymus DNA (CT-DNA) was purchased from Sigma and used without further purification. Tris (hydroxymethyl-aminothane) was purchased from Acros. Sulfosalicylic acid and cupric chloride were of analytical reagent grade purity. The double-distilled deionized water was used throughout. The experiments were carried out at room temperature.

2.2. Methods

DNA stock solution was prepared in double-distilled deionized water and was stored at 4 °C and used not more than 5 days. Monitoring the absorption spectrum and the ratio of the absorbance at 260–280 nm checked the purity of DNA. The ratio being 1.87 indicated that the DNA was fully free of protein [15]. The concentration of DNA in nucleotide phosphate was determined at 260 nm using the absorption coefficient=6600 M^{-1} cm^{-1} [16].

The cyclic voltammetry (CV) was performed an electrochemical analyzer. The three-electrode system (with SCE electrode as a reference, gold disk electrode (diameter 1 mm) as working electrode and platinum as an auxiliary electrode) was used. 50 mmol/l Tris–HCl buffer (pH 7.2) were used as a background electrolyte. The solutions were deoxygenated with nitrogen gas for 15 min prior to measurement.

Potential-controlled DNA cleavage in presence of $Cu(ssal)_2^{2+}$ complex were performed on a gold disk electrode (diameter 5 mm) in a thin layer electrochemical cell which volume was 250 μl. The solution containing 5.0×10^{-4} mol/l $Cu(ssal)_2^{2+}$, 2.0×10^{-4} mol/l DNA and 10 mmol/l Tris–HCl buffer (pH 7.2) was added to the thin layer and electrolyzed at −0.4 V for 30 min. The test solutions were saturated with air except when stated otherwise.

The separation of DNA fragments: after electrolysis being finished, 10 μl electrolyzed solution was taken out by microinjector for chromatographic analysis. The eluent was 10 mmol/l phosphate buffer (pH 7.2) and the flow rate was 1.0 ml/min. A UV–vis spectrophotometric detector was used and the detection wavelength was set at 260 nm, which was the maximum absorption wavelength of DNA. The reproducibility of the chromatographic system was examined by infection DNA solution five times sequentially. The relative standard deviation (RSD) of the DNA chromatograph peak height was less than 5.0%.

3. Results and discussion

3.1. Interaction of $Cu(ssal)_2^{2+}$ complex with DNA

Typical CV curves for $Cu(ssal)_2^{2+}$ complex in the 50 mmol/l Tris–HCl buffer (pH 7.2), in the absence and in the presence of CT-DNA are shown in Fig. 1. In the absence of DNA, $Cu(ssal)_2^{2+}$ complex was reduced to $Cu(ssal)_2^{+}$ complex at the cathodic peak potential, E_{pc}=−165 mV and the anodic peak potential, E_{pa}, appeared at 104 mV. The half-peak width of the cathodic peak, $W_{1/2}$, was 95 mV. The electrode transfer coefficient (α) was 0.65 according to the following equation [17] $W_{1/2}=\frac{62.5}{\alpha n}(n=1)$. The separation of the cathodic and anodic peak potentials, ΔE_p=269 mV, indicated that the electrochemical reaction of $Cu(ssal)_2^{2+}$ complex on the gold electrode was a quasi-reversible redox process. The peak currents(i) were linear with square root of scan rate (v^2) for $v\leq$100 mV/s, indicating that the redox process was controlled by diffusion. Addition of CT-DNA to the $Cu(ssal)_2^{2+}$ complex solution results in a decrease of the anodic and cathodic peak current. It could be explained by a reduction in the apparent diffusion coefficient of

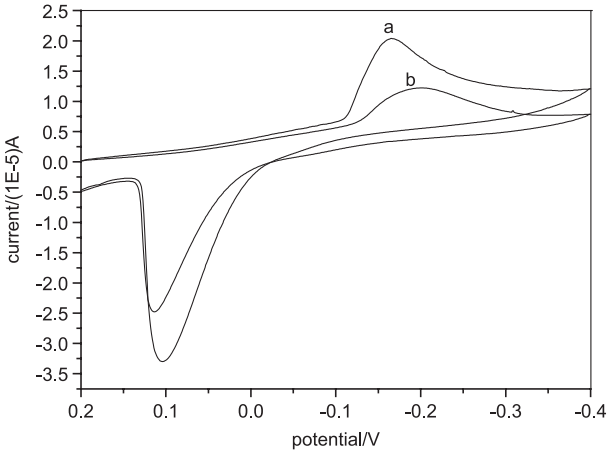

Fig. 1. Cyclic voltammetric curve of 2.0×10^{-3} M $Cu(ssal)_2^{2+}$ complex (a) in the absence and (b) in the presence of 2.0×10^{-3} M DNA.

copper–sulfosalicylic complex at their complexation by the DNA macromolecules [18] and formed DNA–Cu–sulfosalicylic. Those experimental results showed that the interaction occurred between $Cu(ssal)_2^{2+}$ complex and DNA.

3.2. DNA cleavage of electrochemically induced $Cu(ssal)_2^{2+}$ complex

Shown in Fig. 2 are the separation results of the solution which contained 5.0×10^{-4} mol/l $Cu(ssal)_2^{2+}$complex, 2.0×10^{-4} mol/l DNA and 10 mmol/l phosphate buffer (pH 7.2). Fig. 2A is the chromatogram of the solution before electrolysis. A large and broad peak of DNA appeared on the chromatogram at a retention time of 4.54 min. After the solution was electrolyzed 30 min at −0.4 V (vs. Ag/AgCl), the peak of DNA became small and some new chromatographic peaks were observed in Fig. 2B. The retention time

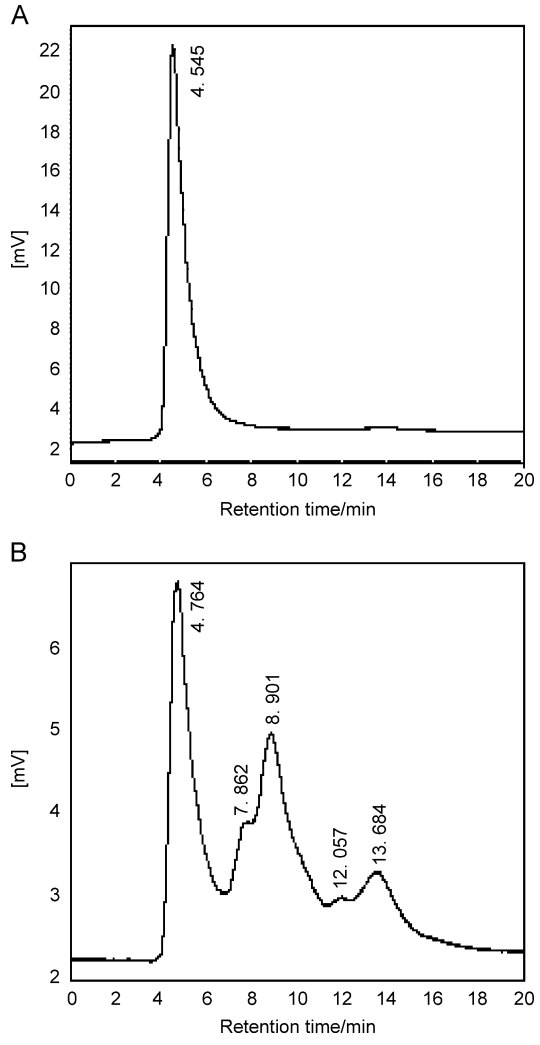

Fig. 2. Chromatograms of the solution containing 5.0×10^{-4} M $Cu(ssal)_2^{2+}$ complex, 2.0×10^{-4} M DNA and 10 mM Tris–HCl buffer (pH 7.2) before (A) electrolysis and (B) after 30 min electrolysis at −0.4 V. Conditions: the eluent 10 mM phosphate buffer (pH 7.2), flow rate 1.0 ml/min.

of these new chromatographic peaks were at 7.86, 8.90, 12.06 min, 13.70 min, respectively. Compared to Fig. 2A, the changes of DNA chromatograms revealed that the structure of DNA molecules obviously varied before and after electrolysis. The long strands of partial DNA molecule break and form short strands fragments of DNA molecules. Hence, there are new peaks occurring on the chromatogram. Under the same experimental condition, the solution without $Cu(ssal)_2^{2+}$ complex was electrolyzed. The chromatograms of DNA changed slightly before and after electrolysis. It revealed that the structure of a small amount of DNA molecules could be destroyed after the solution without $Cu(ssal)_2^{2+}$ complex was electrolyzed. This is because oxygen was reduced electrochemically to short-lifetime radical intermediates (superoxide or hydroxyl) to cause weakly DNA cleavage [14,19]. The above experimental results illustrated that the reason for causing the change of DNA structure was not DNA reacting on the electrode, but electrochemically induced DNA cleavage by the Cu(ssal) complex. For the Shim-pack Diol-300 column was attributed to the size-exclusion chromatographic column, and its separation was based on the size of the molecules separated. In exclusion chromatography, the shorter strand of DNA is, the longer retention time of components is [20,21]. For new peaks appearing at long retention time on the chromatogram, indicating the structure of DNA molecules was destroyed and the strand of partial DNA molecules was broken from the longer to the shorter. The components of retention time at 12.06 and13.70 min were much smaller DNA fragments. The above experimental results indicated that this method of electrochemically induced DNA cleavage by $Cu(ssal)_2^{2+}$ complex was very efficient. The retention time of new chromatographic peaks became longer with increasing electrolysis time. It illustrated that DNA is cleaved into further shorter strand fragments.

In order to explore the mechanism of electrochemically induced DNA cleavage by the $Cu(ssal)_2^{2+}$ complex, the solution with $Cu(ssal)_2^{2+}$ complex was deoxygenated with N_2 before electrolysis. It was not obviously different in the chromatograms of DNA from before electrolysis to after electrolysis. This observation indicated that DNA could not be cleaved by electrochemically induced in the absence of oxygen in solution with $Cu(ssal)_2^{2+}$ complex. In the absence of $Cu(ssal)_2^{2+}$ complex, the chromatogram of DNA changed slightly before and after electrolysis. When hydroxyl radical scavengers, such as DMSO, glycerol, were added to the studied solution, the solution was electrolyzed at the same condition. The results of chromatographic analysis showed that the chromatogram changed slightly before and after electrolysis. It indicated that potential-modulated DNA cleavage by $Cu(ssal)^{2+}$ complex was inhibited by radical scavengers. According to the above experimental results, the mechanism of the cleavage was possibly as follows: the $Cu(ssal)_2^{2+}$ complex was first electrochemically reduced to form $Cu(ssal)_2^{2+}$ complex on the gold electrode surface.

Second, the $Cu(ssal)_2^{2+}$ complex was reoxidized to reform $Cu(ssal)_2^{+}$ complex by the dissolved oxygen, while hydrogen peroxide was generated in the process. It was just hydrogen peroxide initiated a series of reaction to form OH radicals. Third, the formed hydroxyl radical attacked the DNA strand and resulted in the breakage of DNA strand. The processes could be expressed as follows:

$$Cu(ssal)_2^{2+} + e \rightarrow Cu(ssal)_2^{+} \text{ (electrode reaction)}$$

$$2\,Cu(ssal)_2^{+} + 2H^{+} + O_2 \rightarrow 2Cu(ssal)_2^{2+} + H_2O_2$$

$$\text{and } O_2 + e \rightarrow [\cdot O_2^{-}] \rightarrow H_2O_2$$

$$H_2O_2 + 2\,Cu(ssal)_2^{+} \rightarrow OH^{-} + \cdot OH^{-} + 2Cu(ssal)_2^{2+}$$

$$DNA + \cdot OH^{-} \rightarrow DNA \text{ fragments}$$

3.3. Effect of experimental conditions on DNA cleavage

We examined the peak height of DNA at retention time 4.54 min changing with working potential, in order to reveal the influence of working potential on the extent of DNA cleavage. Supporting the peak height of DNA was H and H_i before and after electrolysis, respectively. $(H\text{-}H_i)/H$ would be used for indicating the percentage of DNA cleavage. Fig. 3A shows the percentage of DNA cleavage change with various potentials in the presence of the $Cu(ssal)_2^{2+}$ complex. In the curve, a sharp change at −0.40 V was obtained, and the potential at −0.4 V was chosen in our experiment.

The electrolysis time also affected the percentage of DNA cleavage. The effect of electrolysis time on the percentage of DNA cleavage was shown in Fig. 3B. It can be observed that the percentage of DNA cleavage was enhanced with increasing electrolysis time. The amount of long strand DNA was lessened. However, this change trended to slow. The reason was that, after the solution was electrolyzed for a certain time, the partial long strand DNA was cleaved to form short strand DNA and the amount of short strand DNA increased gradually with increasing electrolysis time. Thus, the probability of long strand DNA attacked by hydroxyl radical was lessened. That the retention time of the cleaved DNA fragments lengthened with increasing electrolysis time indicated these DNA fragments were further cleaved to form much smaller components.

The dependence of percentage of DNA cleavage on the ratio of $Cu(ssal)_2^{2+}$ to DNA concentration was shown in Fig. 3C. It can be seen that when the electrolysis time was controlled for 30 min at −0.4 V, the percentage of DNA cleavage was enhanced with increasing the ratio of $Cu(ssal)_2^{2+}$ complex to DNA concentration. When the ratio was increased to 0.4, the percentage of DNA cleavage was biggest. At low $Cu(ssal)_2^{2+}$ complex concentration, the amount of hydroxyl radical generated in the reaction system was increased with increasing the ratio; the percentage of DNA cleavage was enhanced. When the ratio was increased to 0.4, it is optimum ratio, amounts of the generated radicals depended upon the concentration of dissolved oxygen. Hence, the percentage of DNA cleavage was not further increased.

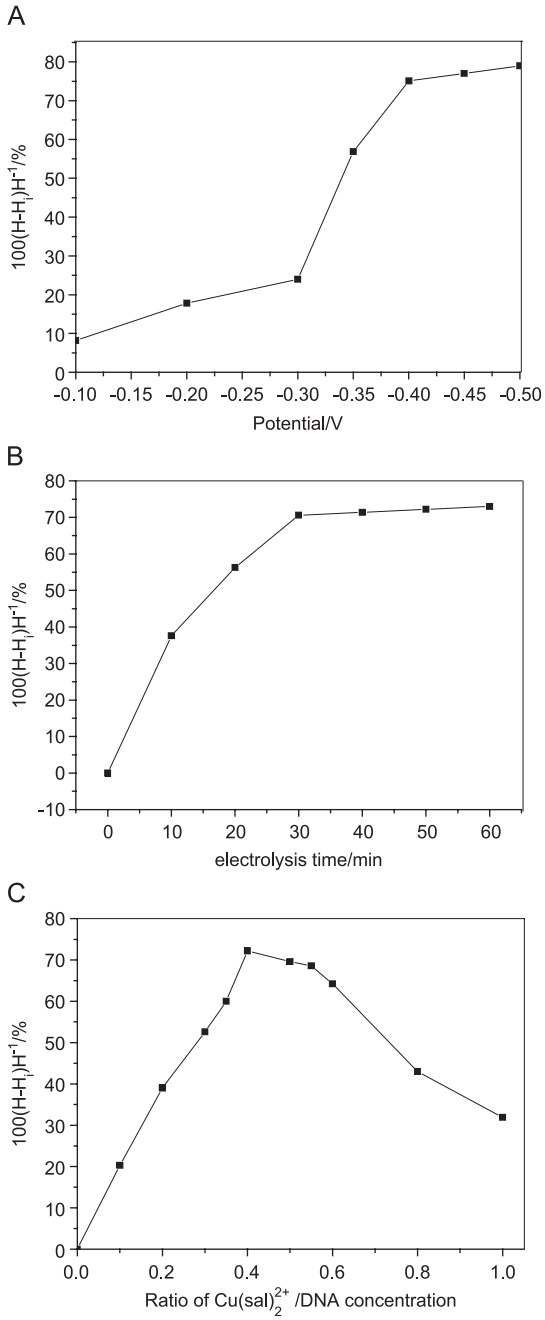

Fig. 3. (A) Dependence of DNA cleavage percentage on working potential: 5.0×10^{-4} M $Cu(ssal)_2^{2+}$ complex, 2.0×10^{-4} M DNA and 10 mM Tris–HCl buffer (pH 7.2). (B) Dependence of DNA cleavage percentage on electrolysis time. 5.0×10^{-4} M $Cu(ssal)_2^{2+}$ complex, 2.0×10^{-4} M DNA and 10 mM Tris–HCl buffer (pH 7.2). (C) Dependence of DNA cleavage percentage on the ratio of $Cu(ssal)_2^{2+}$ complex to DNA concentration. 5.0×10^{-4} M $Cu(ssal)_2^{2+}$ complex, 10 mM Tris–HCl buffer (pH 7.2).

4. Conclusion

In this paper, we show that DNA can be cleaved efficiently by electrochemically controlled copper/sulfosalicylic-mediated Fenton reaction, as well as radical intermediates of oxygen electroreduction can cleave DNA. The extent of DNA cleavage is largely dependent on the working potential, electrolysis time and the concentration of Cu(ssal)$_2^{2+}$ complex. It is possible using this method to simulate the situation in vivo to cleave DNA.

Acknowledgements

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