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Electrochemical Investigation of DNA Binding on Carbaldehyde Oxime by Cyclic Voltammetry

T. R. Ravikumar Naik and H.S. Bhojya Naik[*]

Department of Studies and Research in Industrial Chemistry, School of Chemical Sciences, Kuvempu University, Shankaraghatta-577 451, INDIA.
[*]E-mail: hsb_naik@rediffmail.com

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The electrochemical investigation of interaction of newely synthesized 2-hydroxyquinoline-3-carbaldehyde oxime (HCO) **3a** with calfthymus double-stranded DNA has been investigated by cyclic voltammetric studies at carbon paste electrode (CPE) in Tris-HCl buffer (pH 7.2). It was observed in cyclic voltammetric studies that the curve of HCO solution was higher than solution of HCO and Ds DNA mixture.

Keywords: DNA, cyclic voltametry, carbon paste electrode

1. INTRODUCTION

Electrochemistry has been increasing helpful in elucidating the basic chemistry of biological systems. Substances with reduction potential above 0.5 V (SHE) may undergo electron transfer in vivo [1].

The application of electrochemical methods to the study of organic and metallointeraction to DNA provides a useful complements to the previously used methods of investigation, such as UV-visible spectroscopy, viscosity and circular dichroism studies. Small molecules, which are not amenable to such methods, either because of weak absorption bands or because of overlap of electronic transition with those of the DNA molecules, can potentially, be studied via voltammetric techniques.

DNA biosensor technologies are currently under intense investigation owing to their great promise for rapid and low-cost detection of specific DNA sequences in human, viral and bacterial nucleic acids.

The binding of small molecules to deoxyribonucleic acid (DNA) occurs through primarily in three modes: electrostatic interactions with the negative-charged nucleic sugar-phosphate structure,

binding interactions with two grooves of DNA double helix and intercalation between the stacked base pairs of native DNA [2].

The interactions of some anticancer drugs with DNA have been studied with a variety of techniques [3-6] and, in recent years, there is a growing interest in the electrochemical investigations of interactions between anticancer drugs and other DNA-targeted molecules and DNA [7].

A quantitative understanding of such factors that determine recognition of DNA sites would be valuable in the rational design of new DNA targeted molecules for application in chemotherapy and in the development of tools for biotechnology based on DNA hybridization.

Further, many quinoline derivatives are well known for their various biological activities including anticancer activity [8-14]. Recently, Gopal et al. [15] have reported the DNA binding activities of quinolines derivatives.

In the present study, in continuation of our work on synthesis and DNA binding studies [16-18], we wish to present the interaction of 2-hydroxyquinoline-3-carbaldehyde oxime **(3a)** with double stranded calf thymus DNA (dsDNA) in solution by modified carbon paste electrode (CPE).

2. EXPERIMENTAL PART

2.1. Chemicals

All reagents and solvents were of AR grade, purchased commercially. Tris-HCl buffer salts purchased from Qualigens (India), and Calf thymus DNA (CT-DNA) from Bangalore Gene, Bangalore, India. Tris-HCl buffer (5mM Tris-HCl, 50mM Nacl, pH-7.2, Tris=Tris (hydroxymethyl) amino methane) solution was prepared using deionised double distilled water.

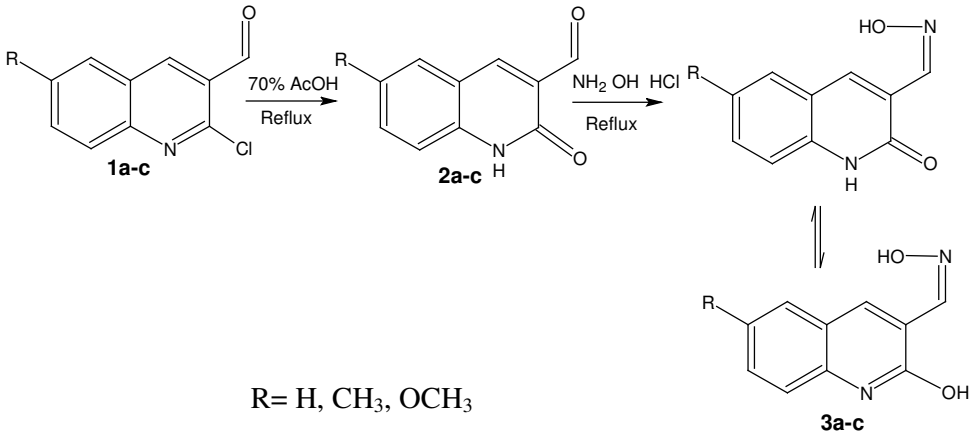
All DNA stock solutions (1000 mg / L) were prepared with buffer solution (50 mM Tris-HCl, 1 mM EDTA, pH 7.2) and kept frozen. More dilute solutions were prepared with 50 mM Tris-HCl, (pH 7.2). The concentration of CT DNA was measured by using its known extinction coefficient at 260 nm (6,600 M^{-1} cm^{-1}) [19]. The absorbance at 260 nm (A_{260}) and at 280 nm (A_{280}) for CT DNA were measured to check its purity. The ratio A_{260} /A_{280} was found to be 1.84, indicating that CT DNA was satisfactorily free from protein. Buffer [1 mM phosphate, pH 7.0, 2 mM NaCl] was used for the DNA binding studies. Stock standard solutions of HCO were prepared by dissolving in 1 mM phosphate (pH 7.0).

2.2. Methods

2.2.1. General procedure for synthesis of 2-hydroxyquinoline-3-carbaldehyde oxime

The 2-chloro-3-formylquinoline **1a** (1.93 g, 0.01 mol) reacts with 70% dilute acetic acid gave **2a.** Then **2a** react with hydroxylamine hydrochloride (2 g, 0.002 mol) in water (5 mL) and sodium hydroxide solution (4N), in presence of ethanol (70 mL) at 75-80 ℃ for one hour with constant stirring. The completion of the reaction was monitored by TLC, eluting the phase ethyl acetate: carbon tetrachloride (60:40). The reaction mixture containing **3a** was cooled, poured into ice-cold water and

acidified with (4N HCl), filtered, washed with 300 mL H_2O and recrystalised using excess alcohol. The same procedure was used for the synthesis of **(3b** and **3c)** compounds. The synthetic scheme and physicochemical data for the synthesized compounds are as shown below.



Scheme. Synthesis of 2-hydroxyquinoline-3-carbaldehyde oxime

2-Hydroxyquinoline-3-carbaldehyde oxime (3a)

Yellow Solid, Yield 83%, m.p.270-273 °C; IR (KBr): N-OH at 3442 cm^{-1}, C=N at 1650 $cm^{-1}$and C=O 1640 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) =12.0 (s, 1H, NH D_2O exchangeable), 9.50 (s, 1H, OH), 7.02-8.10 (m, 5H). Mass, m/z: 190 ($M^{+.2}$), Elemental analysis (%): Calcd. for $C_{10}H_8N_2O_2$: C; 63.82, H; 4.28, N; 14.89 Found: C; 63.80, H; 4.29, N; 14.86.

6-Methyl-3-hydroxy-2-naphthaldehyde oxime (3b)

Yellow Solid, Yield 82%, m.p.268-270 °C; IR (KBr): N-OH at 3440 cm^{-1}, C=N at 1646 $cm^{-1}$and C=O 1646 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) =12.02 (s, 1H, NH D_2O exchangeable), 9.55 (s, 1H, OH), 7.00-8.05 (m, 5H). Mass, m/z: 204 ($M^{+.2}$), Elemental analysis (%): Calcd. for $C_{11}H_{10}N_2O_2$: C; 65.34, H; 4.98, N; 13.85 Found: C; 65.32, H; 4.95, N; 13.84.

6-Methoxy-3-hydroxy-2-naphthaldehyde oxime (3c)

Yellow Solid, Yield 84%, m.p.264-266 °C; IR (KBr): N-OH at 3436 cm^{-1}, C=N at 1655$cm^{-1}$and C=O 1670 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) =11.95 (s, 1H, NH D_2O exchangeable), 9.53 (s, 1H, OH), 7.02-8.06 (m, 5H). Mass, m/z: 220 ($M^{+.2}$), Elemental analysis (%): Calcd, for $C_{11}H_{10}N_2O_3$: C; 50.55, H; 4.62, N; 12.84 Found: C; 50.54, H; 4.60, N; 12.81.

2.3. Cyclic voltammetry

Reduction potentials were earsured by cyclic voltammetry using Electro analyzer-EA 201 fabricated at Bhabha Atomic Research Center (BARC) Bombay, India. Electrochemical cell consists of a glass container with a cap having holes for introducing electrodes and nitrogen. The cell is then maintained oxygen free by passing nitrogen ovr the solution. The reference electrode used is saturated calomel electrode (SCE), which often is isolated from the solution by salt bridge to prevent contamination by leakage from the reference electrode. The auxillary and working electrodes are platinum foil and carbon paste electrode that are placed directly in to the solution.

In a typical cyclic voltammetric experiment of HCO, the reaction mixture consisted of HCO salt in Tris HCl buffer, water and ct-DNA. A stream of nitrogen is passed over them and the reaction mixture was thermostated. The three electrodes were connected to a computer controlled potentiostat and required potential scan rate, current sensitivity, initial potential and final potential are given and the resulting current is measured as a function of applied potential.

2.3.1. Preparation of carbon paste electrode

The carbon paste was prepared in the usual way by hand-mixing graphite power (Aldrich; 1-2 mm) and mineral oil (Sigma). The ratio of graphite powder to mineral oil was 70:30.

The prepared paste was filled in to the teflon well. A Copper wire fixed to a graphite rod and inserted in to the teflon well serves to establish electrical contact with the external circuit. A good reproducibility of electrode response was achieved by simply renewing the surface of carbon paste electrode. New electrode surface was formed by mechanically pressing the carbon paste from the top of the teflon well smoothening of the electrode surface was done by rolling a smooth glass rod on the electrode surface and finally it was cleaned carefully by distilled water.

Each measurement involved fresh carbon paste surface. All the experiments were performed at room temperature (25.0± 0.5) °C

3. RESULTS AND DISCUSSION

The formyl group in quinoline was converted into a oxime (N-OH) group in the presence of hydroxylamine hydrochloride in ethanol at 80-90 °C temperature. The formation of compound was supported spectroscopically by showing the absence of the formyl proton in the ^{1}H NMR spectra at δ10.6 ppm and the appearance of characteristic N-OH absorption at 3442 cm^{-1} and C=N at 1616 cm^{-1} in IR spectra.

Oxygen-nuceophilic substitution was done by the replacement of chlorine using 70% acetic acid which was prepared by literature method [20]. The ^{1}H NMR spectra of compounds **3a** showed at around δ 12-12.02 ppm for N-H proton supporting the hydroxyls tautomeric structure, along with the other aromatic protons.

3.1. Cyclic voltammetric studies

In the cyclic voltammetric (CV) study, the changes in the currents of HCO without and with dsDNA are shown in Figure 1. The results shows that the signal of the bare electrode of HCO was higher than the signal of mixture of HCO-dsDNA on modified CPE.

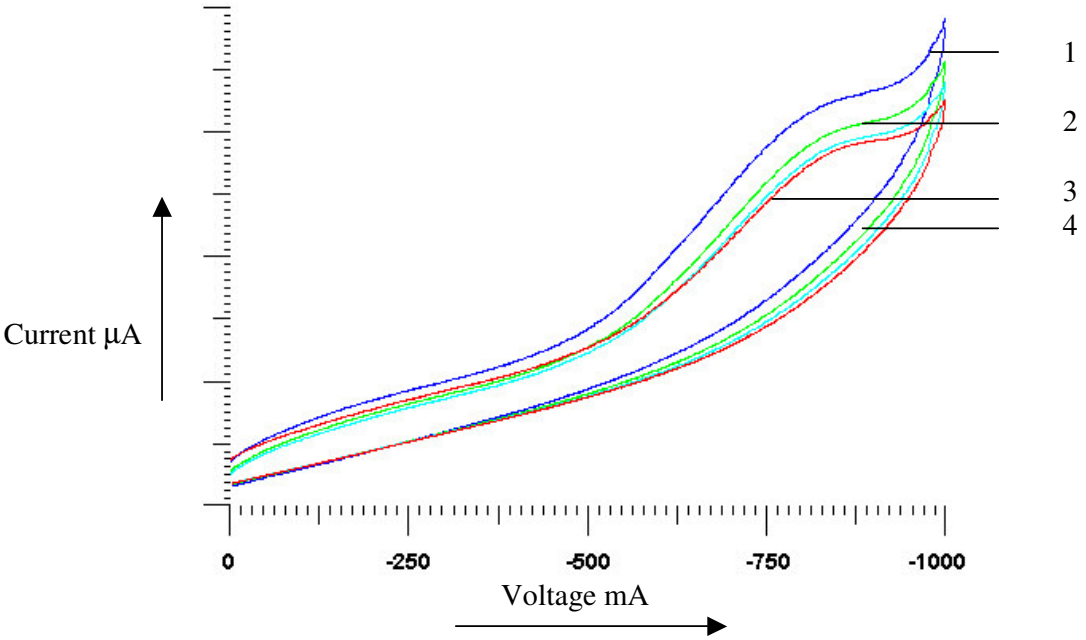


Figure 1. Cyclic voltammograms (CVs) of 0.2 X 10^{-5} M **(3a)** at carbon paste electrode (CPE) in Tris HCl buffer (pH 7.2) in the obsence of DNA (1) and in the presence of 10 µm (2), 20 µm (2), 30 µm (3), 40 µm (4) ct-dsDNA Scan rate: 50 mVs^{-1}.

It was observed that, in recording HCO **(3a)** alone in the forward scan, a single anodic peak was observed, which corresponds to the reduction of **3a** where as in the reverse scan, no anodic peak was observed [21], which indicates that the process is irreversible. When calf thymus dsDNA was added to a solution of **3a**, marked decreases in the peak current heights and shifts of peak potentials to more negative values were observed [22]. Cyclic voltammograms were recorded for 0.1 mM $K_4[Fe(CN)_6]$, which cannot interact with dsDNA in 5 mM phosphate (pH 7.2) at carbon paste in the absence and presence of dsDNA.

3.2. Effect of concentration on DNA binding

As the concentration of ct-DNA increases from 10 to 40 µm, the changes of peak current and potential became slow, the response of HCO decreases sharply with concentration of 40 µm [Table 1, Figure 2]. The cathodic peak decreases from, i_{pc} obtained were found to increase linearly with increase in concentration of ct-DNA. It was also observed that the cathodic peak decreases from -800 mV to -

752 mV. This kind of shift in E_{pc} in cathodic direction with concentration of the ct-DNA molecules observed over the electrode surface suggesting that the **3a** was included completely by dsDNA at the concentration of 40 µm [23]. The cyclic voltammetric behaviors were not nearly affected by the addition of a very large excess of dsDNA (more than 40 µm),

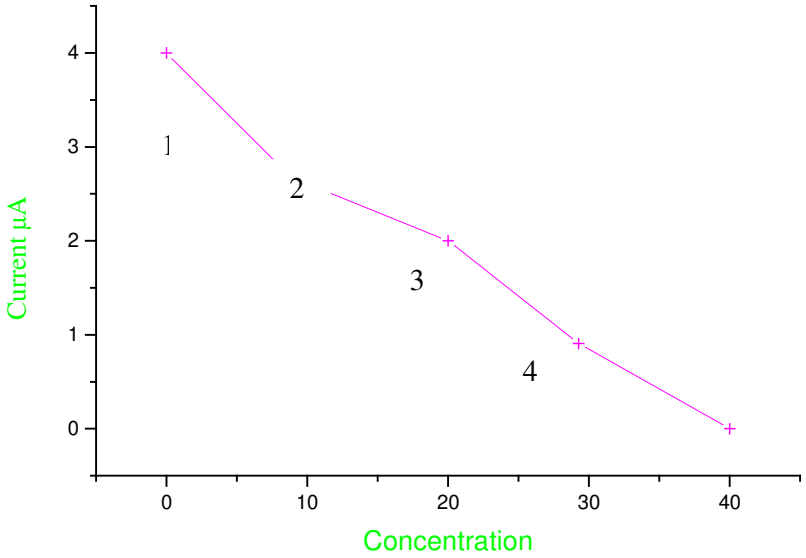


Figure 2. Effect of concentration of HCO with ds DNA at carbon paste electrode (CPE) in Tris HCl buffer (pH 7.2) in the obsence of DNA (1) and in the presence of 10 µm (2), 20 µm (2), 30 µm (3), 40 µm (4) ct-dsDNA Scan rate: 50 mVs^{-1}.

Table 1. Kinetic data obtained for the DNA binding studies of HCO from cyclic voltammetric measurements

Comp(3a)+DNA (µM)	**E_{pc} (V)**	**I_{pc} (µ A)**	**Do (cm^2 s^{-1})**	**K_o (cms^{-1})**
10	-752	24	3.50	70.120
20	-763	28	2.65	55.300
30	-785	32	1.85	21.530
40	-800	36	0.96	9.952

4. CONCLUSIONS

Electrochemical DNA binding study was experimentally convenient and sensitive, so it they required only a small amount of compounds. The present investigation provides strong and impressive evidence for the binding of HCO with ct-DNA.

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