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Research Article

ELECTROCHEMICAL AND ELECTRON PARAMAGNETIC RESONANCE EVIDENCE FOR SEMIQUINONE RADICAL IN THE CLEAVAGE OF CATECHOL BY SCHIFF BASE COMPLEX OF Ru(III) IN AQUEOUS SURFACTANT MICELLE

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ABSTRACT

Binding of 3,5-ditertiarybutyl catechol to Ru(III)(Salen) in aqueous solution of SDS micelle produce $[Ru(III)(Salen)(DTBC)]^-$ adduct. The redox potential of the DTBSQ/DTBC couple was found at -0.234V versus Ag-AgCl reference in aqueous micelles. EPR spectra in aqueous solution and under anaerobic condition show the presence of a semiquinone radical (DTBSQ). The Ru(II)-DTBSQ complex is susceptible to attack by molecular oxygen leading to an extradiol cleavage product which, after further reaction, give 4,6-ditertiarybutyl-2-pyrone as the final product.

Keywords: 3,5-ditertiary butyl catechol, Semiquinone radical, Cyclic voltammogram, Sodiumdodecyl sulphate

INTRODUCTION

Model systems that mimic catechol dioxygenase enzyme reactions have attained considerable significance. The enzymes serve as a part of nature's strategy for degrading aromatic compounds in the environment. They catalyze the oxidation of catechol by breaking the carbon-carbon bond either at C_1-C_2 position (intradiol cleavage) or at C_2-C_3 position (extradiol cleavage). A substrate activation mechanism was proposed for the mechanism for the enzyme reaction wherein coordination of the catechol to the Fe(III) center generates a semiquinone radical which is susceptible for dioxygen attack [1-5]. However, very few of the catechol complexes reported so far as model of the enzyme showing EPR spectral evidence for the semiquinone radical. Recently, there are growing interests in active site models where metal bound phenoxy or semiquinone radicals are stabilized by the hydrophobic environment of proteins. Therefore, experimental evidence for the formation of such radicals in aqueous solutions acquires some interest.

MATERIALS AND METHOD

The present salen ligand was synthesized by condensation of salicylaldehyde and ethylenediamine in 95% ethanol as described by Martell et al. [6,7]. $K[Ru(III)(Salen)Cl_2]$ complex was synthesized according to the reported procedures [6-9]. $RuCl_3.6H_2O$ was purchased from Johnson and Mathey, 3,5-ditertiarybutyl catechol (DTBC) was obtained from Aldrich Chem., and SDS was purchased from Sigma Chemicals Co., USA and used without further purification. Tetramethylammonium bromide was used as counter ions in micelles. All other chemicals were purchased from Aldrich Chem. The pH measurements were done by Elico made model No. L1-10 pH meter. Infra-red spectra were recorded on a Perkin Elmer Spectrum RX-I FT-IR System spectrophotometer. NMR spectra were recorded on a 300 MHz (Advance DPX 300) NMR spectrophotometer using $CDCl_3$ as the solvent. All chemical shifts are reported in ppm relative to an internal standard of tetra methyl silane. EPR spectra were recorded on a Varian E-109, X-band spectrophotometer with 100 KHz field modulation at 27°C in methanol solution using Diphenylpicrylhydrazyl (DPPH) as the calibrating field Marker. A 9.6 GHz, microwave frequency generator was used. Electronic spectra were recorded on a Hitachi U-3210 model double beam spectrophotometer. Cyclic voltametry experiments were carried out with a BAS 100A electrochemical analyzer, Bio-Analytical System, USA. This instrument has an electrochemical cell with a three electrodes system. The working electrode was a glassy carbon electrode (GCE), platinum wire was as an auxillary electrode, and Ag-AgCl electrode was used as a reference electrode. All the cyclic voltammetry experiments were done in an inert atmosphere by purging the solution with N_2 for 15 minutes. Mass spectra were recorded on Perkin Elmer Clarus 600 Gas Chromatograph.

RESULTS AND DISCUSSION

$K[Ru(Salen)Cl_2]$ complex was dissolved in 2% SDS solution at pH 11 and the complex got converted into hydroxo species as $[Ru(III)(Salen)(H_2O)(OH)]$ [10,11]. This complex exhibits an irreversible reduction wave at -0.563V at this high pH value (i.e. pH 11). No oxidation wave is discernible. The irreversible reduction wave corresponds to the Ru(III)/Ru(II) potentials. On adding 1:1 equivalent DTBC to $1.0x10^{-3}$ mole/litre of $[Ru(III)(Salen)(H_2O)(OH)]$ complex, the solution turns green due to formation of $[Ru(III)(Salen)(DTBC)]^-$. The electronic spectrum of this complex exhibits band with λ_{max} at 736 nm, 475 nm and 293 nm.

The model complex $[Ru(III)(Salen)(H_2O)(OH)]$ is coordinative unsaturated; the binding of DTBC is expected to occur without replacing the already bound donors of the Salen ligand. So, the changes in the absorption at 736 nm and 475 nm may be due to structural changes accompanying DTBC binding or to the overlap of ligand→Ru(III) charge transfer bands with phenolate→Ru(III) charge transfer one. The band at 293nm arises presumably from Salen-to-Ru(III) interaction, shifted to higher energy due to the chelation of the $DTBC^{2-}$.

The electronic spectra of $[Ru(III)(Salen)(DTBC)]^-$ comparable to those reported for [Fe(L)(DTBC)]. The visible electronic spectra of $[Ru(III)(Salen)(DTBC)]^-$ and [Fe(L)(DTBC)] shows similarity to those of enzyme-substrate complexes. In the enzyme-substrate complexes the observed spectral change may correspond to displacement of coordinated water or imidazole and not the coordinated tyrosinate without changing in geometry [5,10-13].

The cyclic voltammogram of Ru(III)-DTBC adduct in 2% SDS surfactant micelle (at pH 11) produce the cathodic peak at -0.273V and it is coupled with the anodic peak at -0.199V with a peak potential separation ΔE_p (= E_{pc}-E_{pa}) = 0.078V; cathodic to anodic peak current i_{pc}/i_{pa} = 1.06 at potential scan rate 0.1V/s.

E_{pa} at -0.199V corresponds to the oxidation of coordinated DTBC ligand to coordinated semiquinone [8]. Presence of tertiary butyl blocking groups on the catechol, aromatic ring makes it possible to oxidize DTBC reversible to a stable semiquinone in basic media. The mid-point potential of the coordinated DTBSQ/DTBC is -0.234V (scan rate 0.1 V/s). Scanning to positive potential, oxidizes the coordinated DTBSQ to DTBQ and as a result of an irreversible oxidation wave is observed near +0.204V. Cyclic voltammogram of free DTBC in aqueous 2% SDS surfactant micelle (pH = 11, scan rate 0.1V/s) shows DTBSQ/DTBC and DTBQ/DTBSQ redox potential at -0.172V and +0.524V respectively. The cathodic (i.e. negative) shift of mid-point potential (which is 0.062V cathodic shift) of the coordinated DTBSQ/DTBC redox couple with respect to free DTBSQ/DTBC redox couple indicates that the monodentate chelation of DTBSQ to ruthenium is more stable and reflect the

wstrong affinity of DTBSQ for ruthenium. The mid-point potential also reflects the Lewis acidity of the metal center as modulated by the tetradentate ligand. Similarly, the cathodic shift of the irreversible oxidation wave (which is about 0.320V cathodic shift) of the coordinated DTBQ/DTBSQ with respect to free DTBQ/DTBSQ indicates the stability of DTBQ which does not reduce further. DTBQ formed in this reaction is a poor ligand which does not complex with Ru(II).

The change in voltammograms with increasing the scan rate and the plots of i_{pc} and i_{pa} versus the square root of scan rate ($\nu^{1/2}$) are shown in figure 3(a) and figure 3(b).

The plots of i_{pc} and i_{pa} vs. the square root of scan rate produce a straight line passing through the origin, indicating that the electrode process is diffusion controlled. The ratio of cathodic to the anodic peak current i_{pc}/i_{pa} is almost unity.

This clearly indicates that the redox process associated with the coordinated DTBSQ/DTBC is quasi-reversible. A perusal of Table-1 shows that the mid-point potential ($E_{1/2}$) becomes more cathodic and ΔE_p varies 0.071V to 0.099V with increasing scan rate. Both of these observations are indicative of the quasi-reversible one electron transfer process.

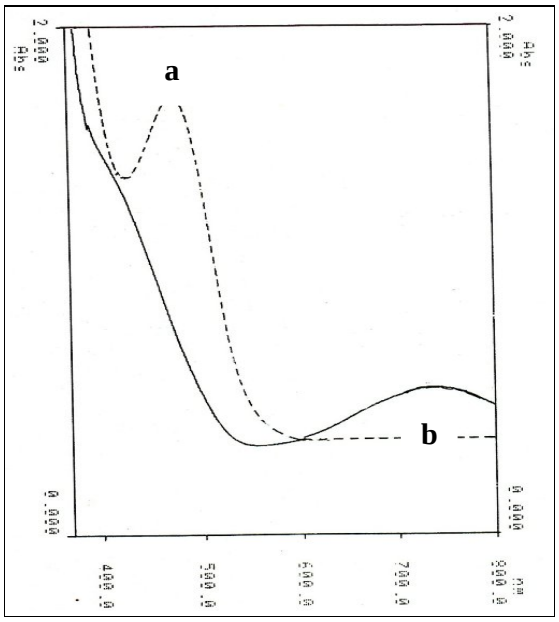
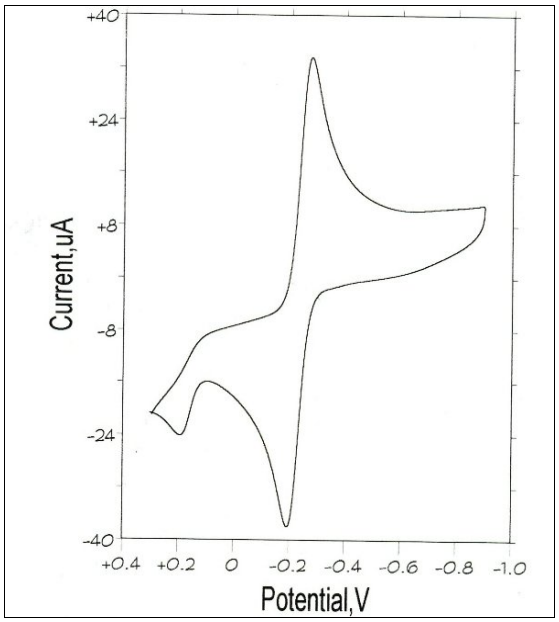


Fig. 1: Electronic spectra of (a) [Ru(III)(salen)DTBC]$^-$ in SDS micelle at pH 11.0 (b) Ru(II)(salen)(DTBSQ) in SDS micelle at pH 11.0 Spectra (b) was recorded of 20 minutes after addition of DTBC



Fig, 2: Cyclic Voltammogramm of [Ru(III)(salen)(DTBC)]$^-$ in SDS micelle; Supporting electrolyte = $NaNO_3$, pH = 11, Temp. = 27^{0}C

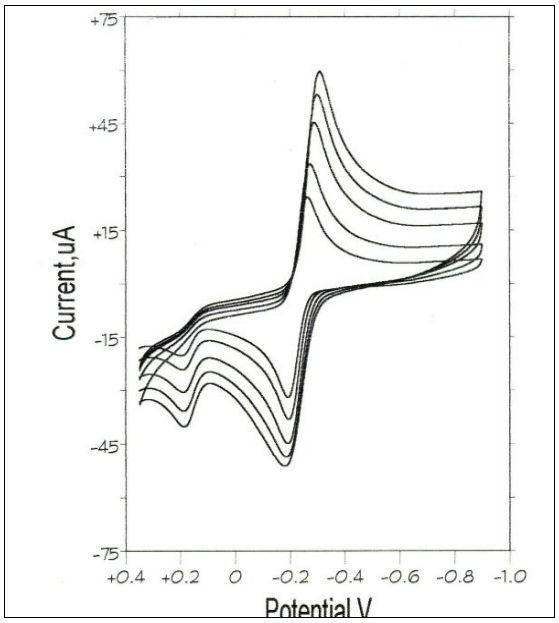


Fig. 3:(a) Cyclic Voltammogram variation for [Ru(III)(salen)(DTBC)]⁻ complex in aqueous SDS micelle with increasing the scan rate at pH 11.0; Scan rates are (i) 0.05V/s (ii) 0.1V/s (iii) 0.2 V/s (iv) 0.3V/s (v) 0.4 V/s

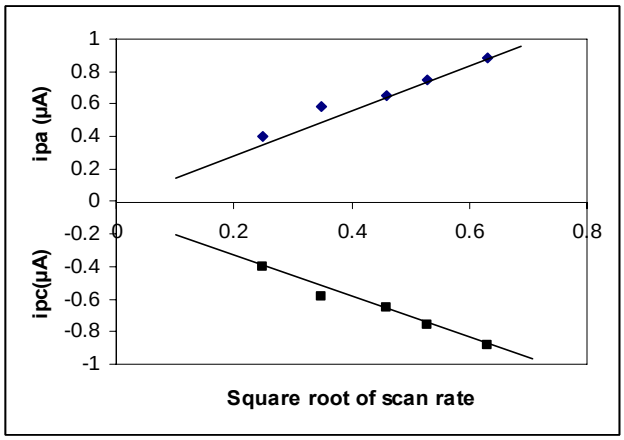


Fig. 3(b): Plots of peak currents i_{pc} and i_{pa} versus the square root of the scan rate for [Ru(III)(salen)(DTBC)]⁻ complex in aqueous SDS micelle at pH 11.

Table 1: Cyclic Voltametric parameters for [Ru(III)(salen)(DTBC)]⁻ in aqueous SDS micelle at micelle at pH 11.0, Temperature 25⁰C

Scan rate (V/s)	E_{pc} (V)	E_{pa}(V)	ΔE_p(V)	i_{pc}/i_{pa}	$E_{1/2}$(V)
0.05	-0.263	-0.192	0.071	0.99	-0.228
0.1	-0.273	-0.195	0.078	1.06	-0234
0.2	-0.286	-0.202	0.084	1.04	-0.244
0.3	-0.290	-0.204	0.086	1.09	-0.247
0.4	-0.309	-0.210	0.099	1.31	-0.260

The EPR spectrum of DTBC adduct of Ru(III)(Salen) complex (figure-4) under anaerobic condition (at pH 11 and 27°C temperature) show two widely separated hyperfine lines (a = ca.3.0 G) followed by further splitting of each of the lines (a_2 = 0.3G). The EPR spectrum in water is typical of that reported for DTBSQ [14,15,16]. The hyperfine coupling was largest for the proton attached to carbon containing the unpaired spin density and further splitting of each line was by interaction with protons of the nearest tertiarybutyl group. Splitting by the other protons is much smaller as was found for DTBSQ in 50% methanol [14,15,16]. The hyperfine lines of the radical are much broader in the micellar solution as compared to that in water; all the lines due to the protons of the tertiary butyl groups are not properly resolved in SDS micelle. This effect could be due to restricted rotation of the entrapped radical in the micelles. On exposure to air the intensity of the EPR signal decreases and the green Ru(II)-DTBSQ complex turns to a brown coloured solution. The radical signal persists for a larger time in the micelles (1-2 hours) as compared to that in water (28 minutes). Similar results are also

obtained with analogous $[Fe(III)(Salen)(DTBC)]^-$ complex in aqueous micellar solutions. The EPR lines are much broader in Fe(II)-DTBSQ complex presumably due to exchange interaction with unpaired electrons at the high-spin Fe(II) ion.

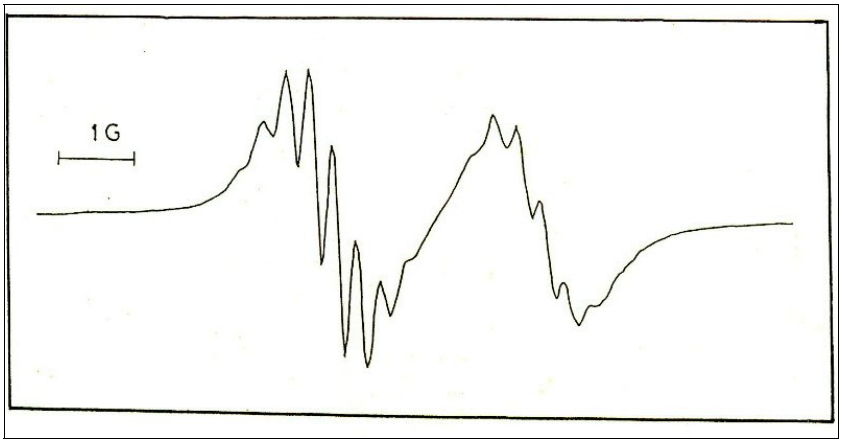
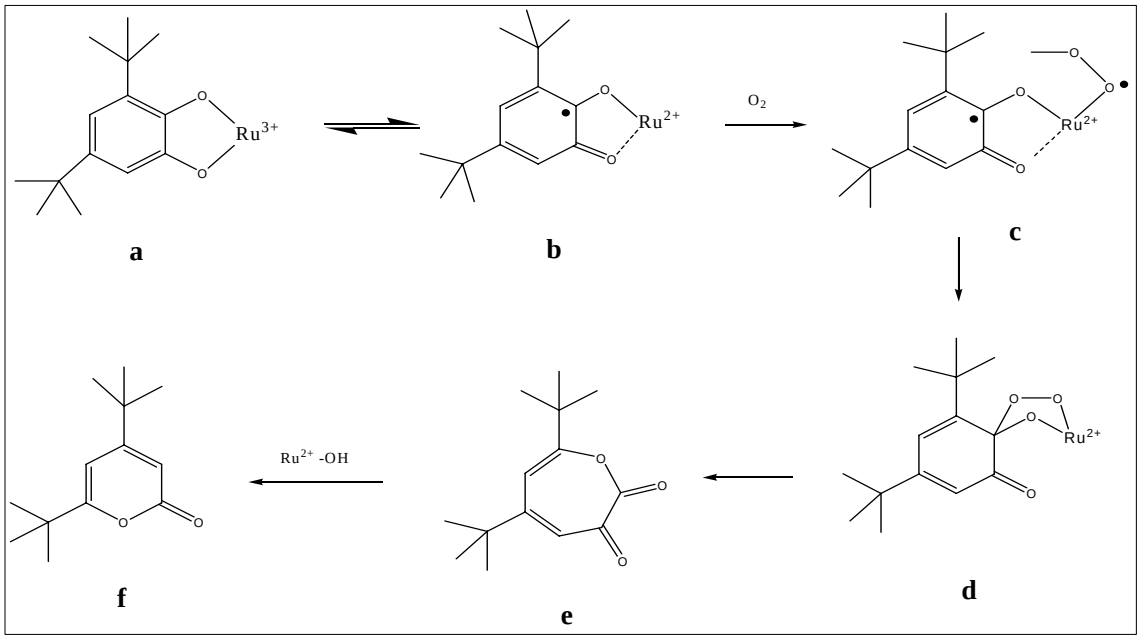


Fig. 4: EPR spectra for Ru(II)(salen)(DTBSQ) in SDS micelle under anaerobic condition at pH 11 and temperature 27^{0}C, Modulation amplitude = 0.16, receive signal = 1.6x10^3, microwave power = 5 mW. Time constant =0.008



Scheme 1: Proposed mechanism for the extradiol cleavage of DTBC

Reactivity with 3,5-ditertiarybutyl catechol

Spectrophotometric study showed that absorbance at 736 nm band $[Fe(III)(Salen)(DTBC)]^-$ adduct gradually decreases on exposure of the solution to air and solution colour turns to red-brown. After complete oxygenation, the micellar solution was acidified with HCl to pH≈3. Organic products were extracted from aqueous micellar solution with hexane and dried over anhydrous Na_2SO_4 and chromatographed on silica gel using chloroform as the eluent [17]. The colorless product was identified by using electronic, infra-red, mass and NMR spectral techniques. The product was identified as 4,6-ditert-butyl-2-pyrone in 55% yield along with DTBQ as a minor product. In a previously study, cleavage of catechol to give 2-pyrone was possible with Cu(II), Fe(III), Ru(II), V(III or IV) complexes.

UV-Vis (in methanol): λ_{max} 276 nm (sharp) and λ_{max} 398 nm.

IR: The compound shows peaks 1741 cm^{-1}, 1666 cm^{-1}, 1569 cm^{-1} and 1240 cm$^{-1.}$ The first of these is assigned to the δ-lactone carbonyl group, the second to the conjugated $\upsilon_{c=c}$ double bonds, third to the frequency originating in the highly conjugated α-pyrone ring.

Mass(C.I. methane): m/e=208(MH^+).

^{1}H NMR ($CDCl_3$): δ1.3, δ0.95($(CH_3)_3C$); δ6.3($(CH_3)_3C$=CH-CO), δ6.9$(CH_3)_3C$=CH-C$(CH_3)_3$.

M.P..=110^{o}C.

The probable binding mode of the Ru(III) complex with 3,5-ditertiarybutyl catechol is depicted in scheme 1. The first step appears to involve the binding of the substrate (state a) followed by formation of semiquinone character of the bound $DTBC^{2-}$ (state b). The Lewis acidity of the ruthenium center enhances the covalence of

the Ru-catecholate interaction and enhances the semiquinone character [14] of the bound $DTBC^{2-}$. This increased semiquinone character renders $DTBC^{2-}$ more prone to oxygen attack and accelerates the of oxidation cleavage [1,3,4]. The attack of O_2 on the activated substrate yield alkyl peroxy radical (state c) which combines with the equally short-lived Ru(II) center to generate an alkyl peroxy-Ru(II) species. Electron transfer from metal to O_2 in Ru(II)-O_2 species results in a super oxide-like moiety (state d) which gives the bound O_2 nucleophilic character. The bound O_2 attacks the carbon adjacent to the enediol unit in a Michael-type addition, to form a peroxy intermediate Decomposition of this peroxy intermediate by Criegee-type rearrangement to afford unstable seven-membered ring keto lactone species (state e). The seven membered ring keto lactone assigned by only from its spectral analysis, though it could not be isolated in pure form because of its instability. The keto lactone decomposes to 4,6-ditertiarybutyl-2-pyrone (state f).

CONCLUSION

The electronic spectra of $[Ru(III)(Salen)(DTBC)]^-$ is comparable with the enzyme-substrate complexes of catechol dioxygenases[14]. Further, the axially coordinated catechol or its derivatives play an important role in determining chemical structure for Ru-Salen complex in biological system for designing the model of catechol dioxygenase enzymes.

The mid-point potential of the coordinated DTBSQ/DTBC redox couple shows large cathodic shift with respect to free DTBSQ/DTBC redox couple. This implies that the monodentate chelation of DTBSQ to ruthenium is more stable. Similarly, the cathodic shift of the irreversible oxidation wave of the coordinated DTBQ/DTBSQ with respect to free DTBQ/DTBSQ indicates the stability of DTBQ. At higher scan rate the coordinated DTBC easily oxidizes to DTBSQ and DTBSQ/DTBC redox process is diffusion controlled quasi-reversible one electron transfer process.

Electron paramagnetic resonance spectroscopy studies supports the evidences of the formation of a short lived intermediate Ru(II)-DTBSQ radical complex before extradiol-cleavage of DTBC in the surfactant micelles. The radical Ru(II)-DTBSQ complex persists for a larger time in the micelles as compared to that in water.

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