Redox Potential of the Active Bleomycin–Fe(III) Complex by Cyclic Voltammetry

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Abstract

The redox potential of the active Fe(III) complex of bleomycin (BLM), which is a DNA cleaving species, was measured by cyclic voltammetry at 25 °C under a hydrogen atmosphere. The cyclic voltammogram showed the reversible one-electron Fe(III)/Fe(II) coupled redox reaction at −0.225 V *versus* SCE. Under the same conditions the redox potentials of the iso-BLM–Fe(III) complex and the deglyco-BLM–Fe(III) complex were also observed, but the cyclic voltammogram for the inactive Fe(III) complex of BLM could not be obtained.

Introduction

Bleomycin (BLM), a glycopeptide antitumor antibiotic, forms a complex with Fe(II) ion [1, 2]. This complex is oxidized easily by air dioxygen to afford an inactive (stable) Fe(III) complex via an active (unstable) Fe(III) complex with DNA cleaving activity [3–9]. It has been demonstrated that the BLM–Fe(II) complex is regenerated from the inactive Fe(III) complex by reduction with reductants such as ascorbate or cysteine [10].

In order to understand the kinetics of DNA degradation, the measurements of the redox potentials of the respective BLM–Fe(III) complexes are of importance. For the inactive BLM–Fe(III) complex, Melnyk *et al.* has found a value of 129 ± 12 mV *versus* NHE by using both microcoulometric and optical absorption methods [11]. In the present paper we show the cyclic voltammogram for the active BLM–Fe(III) complex and for Fe(III) complexes with BLM derivatives, and report the electrochemical relationships between the active and inactive BLM–Fe(III) complexes.

Experimental

BLM and its derivatives were supplied from Nippon Kayaku Co. Ltd., and the BLM–Fe(II) complex (1×10^{-4} M final concentration) was prepared from metal-free BLM in 0.1 M $NaClO_4$ and $FeSO_4$-$(NH_4)_2SO_4 \cdot 6H_2O$ (Mohr's salt) in distilled deionized water under an atmosphere of high grade commercial hydrogen (the hydrogen used at present contains 1 ppm dioxygen). The pH was adjusted to 8.5 with 0.05 M $Na_2B_4O_7$. The inactive (stable) BLM–Fe(III) complex was derived from the BLM–Fe(II) complex by air oxidation. The preparation of the active (unstable) BLM–Fe(III) complex is described in detail in the Results and Discussion section.

Cyclic voltammograms were obtained using a Yanagimoto P-1000H instrument at 25 °C with a mixed solution of 0.1 M $NaClO_4$ and 0.05 M $Na_2B_4O_7$. Cyclic voltammetry was performed with a three-electrode system containing a hanging mercury drop working electrode, a platinum counter electrode and a saturated Calomel reference electrode (SCE). Cyclic voltammograms were recorded at a scan rate of 100 mV s^{-1}. The redox potential ($E_{1/2}$) values were determined as the midpoint between the peak potentials, $E_{1/2} = 1/2(E_{pc} + E_{pa})$. A polarographic experiment was used to check both the potential peaks and the coulometry of the redox couple. ESR spectra were obtained on a JES-FE-2XG spectrometer with 100 KHz magnetic field modulation.

Results and Discussion

It has been reported that different kinds of Fe(III) complexes are obtained from the BLM–Fe(II) complex [8, 9, 12]. The latter complex is transformed spontaneously to the inactive BLM–Fe(III)–OH^-

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complex via the active BLM–Fe(III)–O_2H^- complex by air dioxygen [8, 10].

We could not obtain the cyclic voltammogram for BLM–Fe(III)–OH^-, the inactive (stable) BLM–Fe(III) complex, under an air or nitrogen atmosphere. This result is in accordance with that of cyclic voltammetry and polarography previously reported by Dabrowiak *et al.* [13, 14]. They observed the two-electron reduction of the 4-aminopyrimidine moiety of the BLM ligand at −1.22 V and the two-electron reduction of the bithiazole moiety at −1.48 V. By use of a mediator such as phenazine methosulfate in a coulometric potentiostatic experiment, however, Melnyk *et al.* observed the redox potential of the inactive BLM–Fe(III) complex at $E = 129$ mV *vs.* NHE, and Sugiura *et al.* also estimated the redox $E_{1/2} = 170$ mV by use of the same mediator on the cyclic voltammogram [10].

The species measured in the above two cases appear to be the same inactive BLM–Fe(III) complex. Thus, we tried to detect the redox reaction for the active BLM–Fe(III) complex. Under an atmosphere of hydrogen a brown precipitate, which seems to be a high-spin BLM–Fe(III) complex, was formed in 0.1 M $NaClO_4$ and phosphate buffer (pH 7), but in 0.1 M $NaClO_4$ and sodium borate buffer (pH 8.5) the soluble species was obtained. In order to prepare the latter solution, hydrogen containing 1 ppm dioxygen was bubbled for 30 min through an electrolyte solution which contained BLM (1×10^{-4} M), supporting electrolyte (0.1 M $NaClO_4$) and buffer (0.05 M $Na_2B_4O_7$); Mohr's salt solution (1×10^{-4} M), which had been previously treated with hydrogen, was introduced into the electrolyte solution. After about 1 min of hydrogen bubbling, the cyclic voltammetry was performed. The ESR spectrum of this complex in an electrolytic solution showed the line-shape of the active BLM–Fe(III) complex [8]. As shown in Fig. 1, a reversible cyclic voltammogram with $E_\Delta = 66$ mV and $i_{pc}/i_{pa} = 0.9$ was obtained. A one-electron redox reaction based on the Fe(III)/Fe(II) couple was calculated from coulometry by simultaneous polarographic measurement. The redox potential determined was −225 mV *versus* SCE. In the more negative potential range, two quasi-irreversible redox potentials (E_{pc}) observed at −640 and −850 mV *versus* SCE are suggestive of Fe(II)/Fe(I) and Fe(I)/Fe(0) redox couples, respectively.

Under the same conditions we could measure the cyclic voltammetry for the iso-BLM–Fe(III) and deglyco-BLM–Fe(III) complexes. Iso-BLM is the product of carbamoyl migration in the sugar moiety of BLM [15] and deglyco-BLM is the derivative without the sugar portion of BLM [16]. Their cyclic voltammograms showed nearly reversible one-electron redox reactions. In comparison with the redox potentials for the Cu(II) complexes [17, 18], the values of the Fe(III) complexes are rather similar (see Table I).

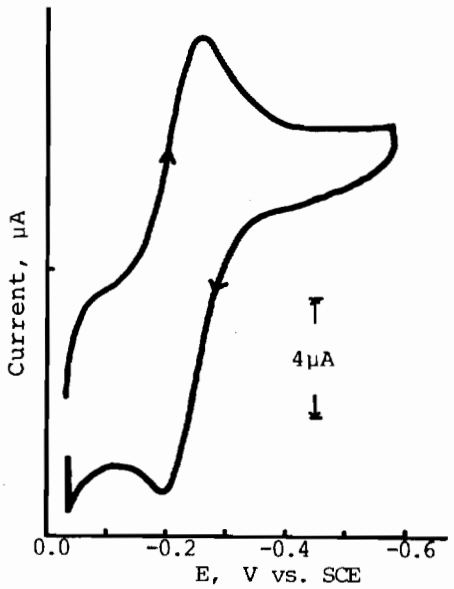

Fig. 1. Cyclic voltammogram of the active BLM–Fe(III) complex in pH 8.5 aqueous solution at a hanging drop mercury electrode.

TABLE I. Redox Potentials (mV *vs.* SCE) of Fe(III) and Cu(II) Complexes for BLM, iso-BLM and deglyco-BLM

	Fe(III)	Cu(II)[a]
BLM	−225	−568
Iso-BLM	−207	−456
Deglyco-BLM	−241	−421

[a] Data from refs. 17 and 18.

These similar values suggest no or negligible interaction between Fe(III) and the sugar portion in the BLM–Fe(III) complex.

The $E_{1/2}$ value (−225 mV) for the active BLM–Fe(III) complex is of the order expected for the cyclic function of the BLM–Fe system [10]. The active BLM–Fe(III) complex was more stable (108 mV) than that of the inactive BLM–Fe(III) complex reported by Melnyk *et al.* This situation suggests that the inactive BLM–Fe(III) complex is more easily reduced than the active BLM–Fe(III) complex by reductants such as glutathione ($E = -230$ mV) and NADH ($E = -318$ mV). Hydrogen peroxide (H_2O_2) ($E = 270$ mV) cannot reduce both BLM–Fe(III) complexes to the BLM–Fe(II) complex. However, by addition of H_2O_2 to the inactive BLM–Fe(III) complex, the active BLM–Fe(III) complex (which is formed from BLM–Fe(II) and O_2) is regenerated. Interestingly, the BLM–Cu(II) complex, which has a more negative redox potential [17, 18], generates radicals such as $\cdot OH$ and $O_2^{\cdot-}$ with H_2O_2 (unpublished data). These facts may be understood by considering

both the small difference in energy levels between the redox potentials of the active and inactive BLM–Fe(III) complexes and the electron negative value for the active BLM–Fe(III) complex, as demonstrated here.

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