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RESEARCH ARTICLE

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Electrochemical studies of reaction of ciprofloxcin and DNA

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Abstract The electrochemical behavior of ciprofloxacin (CFX) and its interaction with the natural calf thymus DNA (ctDNA) is studied by using pulse difference voltammetry on a carbon electrode. CFX shows a well-defined oxidative peak at+0.88 V. As a result of reaction with ctDNA,the oxidative peak of CFX decreased markedly. According to the electrochemical equation deduced in this paper, the binding constant of 1.36×10^5 $(mol/L)^{-1}$ and the binding size of 1.94 (base pairs) of CFX with ctDNA were obtained by nonlinear fit analysis of the electrochemical data. The mechanism of the interaction was explored.

Keywords ciprofloxacin, DNA, electrochemical method, interaction

1 Introduction

Quinolones are an important group of antibiotics and several quinolones are in common clinical use [1]. This fact motivated studies on quinolones. More than 15000 articles on quinolones have been published since 1982 [1]. Fluorescence [2–4], HPLC [5–7], NMR [1], and X-ray crystallography [8] techniques have been used to study the interactions of the quinolone-DNA complex. But the electroanalytical method for CFX with a non-mercury electrode has not, as of yet, been reported [9–16].

In this paper, the electrochemical studies on the interaction of CFX with DNA at a waxed graphite electrode are reported and new electrochemical equations based on statistical arguments [17,18] are presented in which the cooperative parameters of targeting molecules is introduced to describe both non-cooperative and cooperative binding of targeting molecules to DNA. Based on the new equations, the binding constant (K) of DNA-targeting molecule complex and the binding site size (s) can be obtained conveniently by non-linear fit analysis of the experimental data through voltammetry. Our works will provide a useful aid to study binding quinolones with DNA.

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2 Theory

Model: (i) The DNA molecule is a one-dimensional homogeneous receptor. It is taken as a linear array of L identical repeating units, and the basic receptor unit corresponds to a nucleic acid base-pair or backbone phosphate. Furthermore, we define it as a polar receptor and oriented in $3' \rightarrow 5'$ from left to right, for a single-stranded nucleic acid. (ii) The DNA targets are regarded as ligands. The ligand is also assumed to be polar and assumed to bind with a fixed orientation with respect to the receptor. A ligand molecule is assumed to bind to the receptor and to cover s consecutive receptor units. s is named the binding site size. Initially we also consider the receptor to be infinitely long, operationally this means that if $L >> s$, the end-effects can be neglected; (iii) The effects among the ligands were not considered; (iv) The bare receptor units which were not covered by ligands were named f and the receptor units which were covered by ligands were named b. If an electroactive molecule ET (the total concentration of ligands is C_t (mol/L), the concentration of binding ligands is C_b (mol/L) and the concentration of free ligands is C_f (mol/L)) reacts with DNA at a binding site, which is composed of s bases or base pairs (the concentration of receptors is C_{DNA} (mol/L) and the concentration of receptors in units is C_{bP} (mol/L)), the compound-DNA is named as ET-DNA. For which the mass-action equations to the binding type is:

$$C_b / C_f = K C_s \tag{1}$$

And the average number of bound ligands per receptor under a particular set of experimental conditions is Z. we obtain:

$$C_b = ZC_{DNA} \quad (2)$$

Q is the number of binding site and

$$C_s = QC_{DNA} \quad (3)$$

Eqs. (2) and (4) were brought to Eq. (1), and dividing both sides by C_{DNA}, we obtain:

$$Z/C_f = KQ \quad (4)$$

For Z ligands bound to the receptor, there are $(Z+1)$ gaps (counting all gaps where $g \geqslant 0$). Using conditional probabilities we derive an expression for the probability, P_g, that any particular gap between two bound ligands is exactly g free receptor units long.

$$p_g = (b_s f)(ff)^{(g-1)}(fb_1) \quad (5)$$

Here (fb_1) is the probability, given a free receptor unit (i.e. an f unit), that the left end of a bound ligand (i.e. a b_1 unit) lies on the immediate right. (ff) is the probability, given a free receptor unit (i.e. an f unit), that another free receptor unit (i.e. a f unit) lies on the immediate right and $(b_s f)$ is the probability, given the right end of a bound ligand (i.e. a b_s unit), that a free receptor unit (i. e. an f unit) lies on the immediate right. We next obtain an expression for the average number of each of the three types of free binding sites per gap in terms of P_g and the following considerations: for $g < s$, there are no free binding sites; for $g = s$, there is one doubly contiguous binding site; for $g > s$,

$$S = \sum_{g=s}^{\infty}(g-s-1)P_g = (b_s f)(ff)^{s-1}/(fb_1) \quad (6)$$

When L goes toward infinity, $Z+1 \approx Z$, hence the average number of each of the three types of free binding sites per receptor are:

$$Q = (Z+1)\cdot S \approx Z\cdot S \quad (7)$$

And we obtain:

$$1/C_f = K\cdot(b_s f)\cdot(ff)^{s-1}/(fb_1) \quad (8)$$

based on these supposedly different conditional probabilities that can be expressed by the Eq. (9) and Eq. (10).

$$(ff) + (fb_1) = 1 \quad (9)$$

$$(b_s f) + (b_s b_1) = 1 \quad (10)$$

Here $(b_s b_1)$: the probability, given the right end of a bound ligand (i.e. a b_s unit), that the left end of a bound ligand (i.e. a b_1 unit) lies on the immediate right.

Consider a point on the equilibrium binding isotherm at which there are, on the average, Z ligands bound to the receptor. The total number of covered receptor units is then (sZ), and the fraction is sZ/L. It can also be represented as sC_b/C_{bp}. Hence the fraction of all receptor units which are free is $(1 - sC_b/C_{bp})$.

However, due to the nature of the lattice, there are only two ways in which this two-step random selection can be made. Either the first unit chosen is free (an f unit chosen with probability $(1 - sC_b/C_{bp})$) and has a free unit to its right (conditional probability, ff); or the first unit chosen is the right end of a bound ligand (a b_s unit chosen with probability $sC_b/C_{bp}/s = C_b/C_{bp}$ and has a free unit to its right (conditional probability $b_s f$). Since the over-all probability that the second unit is free must be independent of the method of (random) selection, we obtain:

$$(1 - sC_b/C_{bp})(ff) + C_b/C_{bp}(b_s f) = 1 - sC_b/C_{bp} \quad (11)$$

By rearranging terms among Eqs. (9), (10) and (11), and solving a quadratic, we get the following expressions for the conditional probabilities:

$$(ff) = (b_s f) = \frac{1 - sC_b/C_{bp}}{1 - (s-1)C_b/C_{bp}} \quad (12a)$$

$$(fb_1) = (b_s b_1) = \frac{C_b/C_{bp}}{1 - (s-1)C_b/C_{bp}} \quad (12b)$$

Substituting Eq. (12a) and (12b) into Eq. (8), we get the binding equation:

$$\frac{C_b/C_{bp}}{C_f} = K(1 - sC_b/C_{bp})\cdot\frac{1 - sC_b/C_{bp}}{1 - (s-1)C_b/C_{bp}} \quad (13)$$

Let us assume that free ligands and bound ligands in the binding process are electroactive. It is reasonable to assume that both free and bound ligands will undergo similar electrode reactions in the same potential region. i_{pf} is the current of the totally free ligand (μA); i_{pf} is the current of the totally binding ligand (μA); i_p is the current actually measured (μA); k_f and k_b are the heterogeneous rate constants of the bound ligands and the free ligands at a fixed potential, respectively, and x is the fraction of bound ligands. When enough receptors are added, the current is primarily attributed to bound ligands:

$$i_{pb} = k_b C_t \quad (14)$$

and the total contribution to current is from free ligands when no receptors are added:

$$i_{\mathrm{pf}} = k_{\mathrm{f}} C_{\mathrm{t}} \tag{15}$$

The fraction of bound ligands can be described as:

$$x = (i_{\mathrm{pf}} - i_{\mathrm{p}})/(i_{\mathrm{pf}} - i_{\mathrm{pb}}) \tag{16}$$

According to Eqs. (14) – (16), we obtain:

$$C_{\mathrm{f}} = (1-x)C_{\mathrm{t}} = \frac{(i_{\mathrm{p}} - i_{\mathrm{pb}})C_{\mathrm{t}}}{i_{\mathrm{pf}} - i_{\mathrm{pb}}} \tag{17}$$

$$C_{\mathrm{b}}/C_{\mathrm{bp}} = xC_{\mathrm{t}}/C_{\mathrm{bp}} = \frac{(i_{\mathrm{pf}} - i_{\mathrm{p}})C_{\mathrm{t}}}{(i_{\mathrm{pf}} - i_{\mathrm{pb}})C_{\mathrm{bp}}} \tag{18}$$

$$\frac{C_{\mathrm{b}}/C_{\mathrm{bp}}}{C_{\mathrm{f}}} = \frac{(i_{\mathrm{pf}} - i_{\mathrm{p}})}{(i_{\mathrm{p}} - i_{\mathrm{pb}})C_{\mathrm{bp}}} \tag{19}$$

Both Eq. (18) and Eq. (19) substitute in Eq. (13), we obtain a new electrochemical equation:

$$\frac{(i_{\mathrm{pf}} - i_{\mathrm{p}})}{(i_{\mathrm{p}} - i_{\mathrm{pb}})C_{\mathrm{bp}}} = K\left(1 - s\frac{(i_{\mathrm{pf}} - i_{\mathrm{p}})C_{\mathrm{t}}}{(i_{\mathrm{pf}} - i_{\mathrm{pb}})C_{\mathrm{bp}}}\right) \cdot \left(\frac{1 - s\frac{(i_{\mathrm{pf}} - i_{\mathrm{p}})C_{\mathrm{t}}}{(i_{\mathrm{pf}} - i_{\mathrm{pb}})C_{\mathrm{bp}}}}{1-(s-1)\frac{(i_{\mathrm{pf}} - i_{\mathrm{p}})C_{\mathrm{t}}}{(i_{\mathrm{pf}} - i_{\mathrm{pb}})C_{\mathrm{bp}}}}\right)^{s-1} \tag{20}$$

i_{pf}, i_{pb}, i_{p} can be obtained by electrochemical method and k, s can be obtained by nonlinear fit analysis according to the Eq. (20).

3 Experimental

3.1 Reagents

Calf thymus DNA (ctDNA) was obtained from the Sigma Chemical Company and the stock solution was prepared by dissolving it in triple distilled water. Solutions of DNA (*ca*. 10^{-5} mol/L in nucleotide phosphate, NP) gave ratios of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of *ca*. 1.8–1.9, indicating that the DNA was sufficiently free of protein. Stock solutions (the concentrations determined by the absorbance at 260 nm in nucleotide phosphate) were stored at 0–4°C and used up in 3 days. CFX was also obtained from Sigma Chemical Company and the stock solution was prepared by directly dissolving it in 0.01 mol/L NaCl-tris buffer (pH 7.2–7.3), storing it at 0°C, then using it up in three days.

All other chemicals used were analytical reagent grade. Triply distilled water was used in all solutions.

3.2 Instrumentation

All electrochemical experiments were carried out in a CHI660A electrochemical analyzer (Bioanalytical Systems, USA). The working electrode was a waxed graphite one with a geometric area of 0.283 cm^2. An Ag/AgCl electrode was used as reference electrode, and a platinum wire as counter electrode.

A UV-visible measurement was performed on the Shimadzu UV-265 record spectrophotometer.

3.3 Process

All voltammetric experiments were performed in 5–10 mL cells. The supporting electrolyte is 0.01 mol/L NaCl-tris solution.

Free CFX solutions: 0 mL, 0.1 mL, 0.2 mL, 0.4 mL, 0.8 mL, 1.0 mL 2.0 × 10^{-4} mol/L CFX were added serially in 5 mL cells, then 4 mL, 3.9 mL, 3.8 mL, 3.6 mL, 3.2 mL and 3.0 mL. 0.05 mol/L NaCl-Tris was added to in turn. So the overall volume is 4 mL in every cell. These solutions were then stirred .

Binding CFX solutions: 0 mL, 0.1 mL, 0.2 mL, 0.4 mL, 0.8 mL, 1.0 mL 2.0 × 10^{-4} mol/L CFX were added serially in 5 mL cells, then 4 mL, 3.9 mL, 3.8 mL, 3.6 mL, 3.2 mL and 3.0 mL. 2.457 × 10^{-3} mol/L ctDNA was added to in turn. So the overall volume was 4 mL in every cell. These were mixed for 1 h by stirring.

Both free and binding CFX solutions: 0.5 mL 4.626 × 10^{-4} mol/L ctDNA were added in every cell, then 0 mL, 0.1 mL, 0.15 mL, 0.2 mL, 0.25 mL, 0.3 mL, 0.35 mL, 0.4 mL, 0.5 mL, 0.6 mL, 0.7 mL, 0.8 mL, 0.9 mL, 1.0 ml 2.0 × 10^{-4} mol/L CFX and 3.5 mL, 3.4 mL, 3.35 mL, 3.3 mL, 3.25 mL, 3.2 mL, 3.15 mL, 3.1 mL, 3.0 mL, 2.9 mL, 2.8 mL, 2.7 mL, 2.6 mL, 2.5 mL 0.01 mol/L NaCl-tris are added in turn. The overall volume is 4 mL and these were mixed for 1 h by stirring.

A series of solutions were measured by pulse difference voltammetry (DPV) (Pulse Width was 50 ms). Non-linear fit analysis of experimental data based on the deduced equation presented above to obtain K and s was performed using ORGIN 5.0 software. All experiments were carried out at the ambient temperature of the laboratory (23–27°C). The electrochemical data, unless specified otherwise, were the average of at least three to five replicate measurements.

4 Results and discussion

4.1 The electrochemical behavior of CFX at a waxed graphite electrode

CFX in 0.01 mol/L NaCl-Tris buffer (pH 7.2–7.3) shows an oxidation process at a waxed graphite electrode

(Fig. 1a). From the literature [17] we know that the CFX oxidation is an irreversible process. The oxidation peak may be attributed to the irreversible oxidation of the piperazine moiety of CFX molecule, in accordance with the redox mechanism postulated by Kauffmann et al. [17].

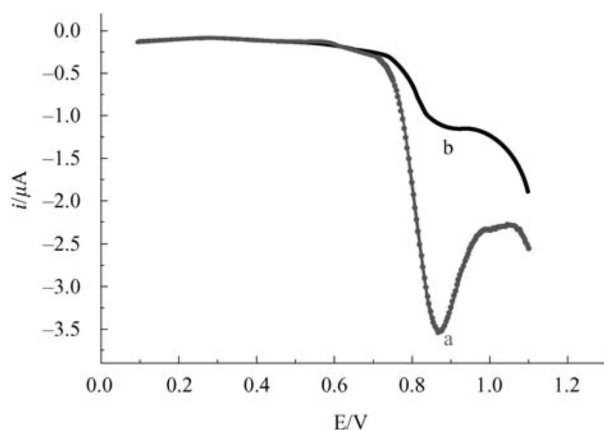

Fig. 1 Different pulse voltammograms of CFX interaction with ctDNA
Support solution: 0.01 mol/L NaCl-tris (pH 7.2); Scan rate: 20 mV/s; Pulse width: 50 ms
a) 2.0×10^{-5} mol/L CFX; b) 2.0×10^{-5} mol/L CFX+1.0×10^{-3} mol/L ctDNA

4.2 Interaction of CFX and DNA

In the present DNA, the peak of CFX sharply decreases in peak current but the peak potential does not shift (Fig. 1b). As shown in Fig. 2, the i_p values show a linear shift with c (free CFX or binding CFX solution) over the range of $5.0 \times 10^{-6} - 5.0 \times 10^{-5}$ mol/L.

Because i_p values were linearly dependent on c for either free CFX or binding CFX solution, we determined the peak currents of the free and binding CFX solution. The results are given in Table 1.

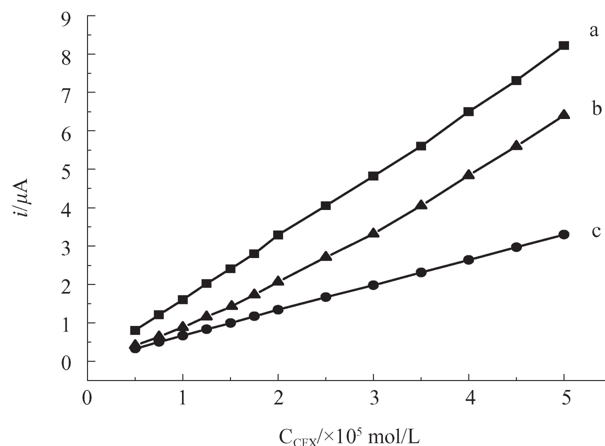

Fig. 2 Calibration curve
a) CFX; b) CFX+2.0×10^{-4} mol/L ctDNA; c) CFX+2.0×10^{-3} mol/L ctDNA

Based on the data in Table 1, we obtain a series of values of $\frac{(i_{pf} - i_p)}{(i_p - i_{pb})C_{bp}}$. According to Eq. (20), we obtained the curves of CFX and ctDNA to see if it was cooperative in CFX (shown in Fig. 3). The binding constant of 1.36×10^5 (mol/L)$^{-1}$ and the binding size of 1.94 (base pairs) of CFX with ctDNA were obtained by nonlinear fit analysis of electrochemical data.

5 Conclusion

In this paper, a new equation is described based on conditional probabilities. The binding constant (K) and binding site size (s) could be obtained by nonlinear fit analysis of the experimental data. It shows that the electrochemical reach for this kind of interaction is sensitive, fast and economical. The equation offers a great promise for the interaction of DNA and other electroactive molecules and for the mechanism of action.

Table 1 Relationships between peak current and concentration of CFX and ctDNA

$C_t/10^{-5}$ mol L^{-1}	i_{pf}/μA	adding C_{DNA} /10^{-5} mol L^{-1} (non-saturation)	i_p/μA	adding C_{DNA} /10^{-5} mol L^{-1} (saturation)	i_{pb}/μA	$\frac{(i_{pf}-i_p)C_t}{(i_{pf}-i_{pb})C_{bp}}$	$\frac{(i_{pf}-i_p)}{(i_p-i_{pb})C_{bp}}$
0.50	0.8035	4.038	0.4140	2.396×10^{-3}	0.3306	0.09178	1.041×10^5
0.75	1.205	4.038	0.6412	2.365×10^{-3}	0.501	0.13386	8.962×10^4
1.00	1.600	4.038	0.8849	2.334×10^{-3}	0.6676	0.17093	7.334×10^4
1.25	2.023	4.038	1.159	2.303×10^{-3}	0.8345	0.20252	5.934×10^4
1.50	2.412	4.038	1.423	2.273×10^{-3}	0.9918	0.23266	5.101×10^4
1.75	2.801	4.038	1.733	2.242×10^{-3}	1.170	0.25541	4.229×10^4
2.00	3.286	4.038	2.068	2.211×10^{-3}	1.341	0.27913	3.734×10^4
2.50	4.046	4.038	2.705	2.149×10^{-3}	1.669	0.31433	2.885×10^4
3.00	4.824	4.038	3.322	2.088×10^{-3}	1.982	0.35347	2.499×10^4
3.50	5.602	4.038	4.409	2.027×10^{-3}	2.310	0.36798	1.990×10^4
4.00	6.497	4.038	4.844	1.966×10^{-3}	2.640	0.38206	1.671×10^4
4.50	7.309	4.038	5.602	1.904×10^{-3}	2.971	0.39455	1.445×10^4
5.00	8.215	4.038	6.349	1.843×10^{-3}	3.301	0.41218	1.308×10^4

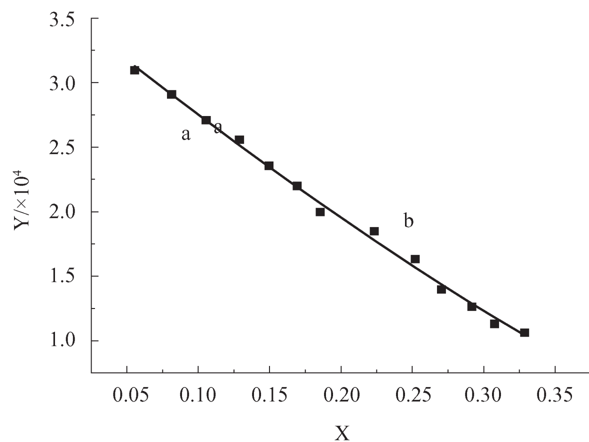

Fig. 3 Binding current of CFX with ctDNA in 0.01 mol/L NaCl-Tris solution $X=\frac{(i_{\mathrm{pf}}-i_{\mathrm{p}})C_{\mathrm{t}}}{(i_{\mathrm{pf}}-i_{\mathrm{p}b})C_{\mathrm{bp}}}$; $Y=\frac{(i_{\mathrm{pf}}-i_{\mathrm{p}})}{(i_{\mathrm{p}}-i_{\mathrm{pb}})C_{\mathrm{bp}}}$

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