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Interaction of anticancer herbal drug berberine with DNA immobilized on the glassy carbon electrode

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ABSTRACT

The interaction of anticancer herbal drug berberine with double-strand DNA (dsDNA) and single-strand DNA (ssDNA) in solution, dsDNA immobilized on the glassy carbon electrode prepared by Langmuir–Blodgett technique, were investigated by electrochemical techniques (cyclic voltammetry, differential pulse voltammetry) and UV spectroscopy. The presence of DNA results in a decrease of the currents and a negative shift of the electrode potentials from the DPV curves of berberine, indicating the dominance of electrostatic interactions. The spectroscopy data confirmed that the predominant interaction between berberine and DNA is electrostatic. The binding of berberine with DNA, when analyzed in terms of the cooperative Hill model, yields the binding constant $K_a = 2.2(\pm 0.2) \times 10^4\ M^{-1}$, corresponding to the dissociation equilibrium constant $K_d = 4.6(\pm 0.3) \times 10^{-5}$ M, which in the range of the applied concentrations of DNA (bp) and berberine, and a Hill coefficient $m = 1.82(\pm 0.08)$ in Britton–Robinson buffer solution (0.05 M, pH 5.72) at $T = 298$ K (25 °C). Apparently, at least two molecules of berberine have to bind as a couple to cause, e.g., the "elementary event" of current change. The results are suggestive for further fruitful applications of this anticancer herbal drug and DNA-modified electrodes.

1. Introduction

Herbal medicine plays an important role in clinical therapy and is widely accepted as a potential high quality pool for drug screening, owing to its unmatched chemical diversity and minimum side effects. Berberine was initially isolated from the herbs *Rhizoma coptidis* (Huang-Lian), belonging to the camptothecin family of drugs. For the chemical structure of berberine, see Fig. 1. Berberine is known as an important compound in cancer therapy, possessing anticancer activity in vitro and in vivo [1].

The interaction of small molecules with DNA plays an important role in life phenomena, such as mutations of genetic information leading to diseases, by causing changes in replication and transcription of DNA. On the other hand, the analytical results carry information for molecular recognition in DNA hybridization and for sensing of bioactive species, such as anticancer drugs, usefulness also in clinical diagnostics and general biomedicine.

The Langmuir–Blodgett (LB) technique has attractive features for the fabrication of molecular assemblies with controlled thickness at molecular dimensions and a well-defined molecular orientation [2]. The LB technique continues to make great contributions to the further development of DNA-modified electrodes for analytical sensing.

Electrochemical techniques have attracted appreciable attention due to the inherent specificity and sensitivity. Simple and low cost devices and easy manipulation facilitate to investigate interaction mechanisms and dynamics of the interactions between DNA and other compounds [3,4], also in the living cells in vivo [5]. There is continuous interest in the electrochemical interactions of anticancer drugs and other molecules with DNA [6–11].

In this work, electrochemical data are reported on the interaction between berberine and DNA, obtained by applying cyclic voltammetry (CV) and differential pulse voltammetry (DPV) to DNA-modified glassy carbon electrode (GCE). The results suggest that the principal interactions between the cationic berberine and the polyanionic DNA are cooperative and dominant of electrostatic nature.

2. Experimental

2.1. Apparatus and reagents

Model 650A electrochemical analyzer (CHI Instrument Company, USA) served to perform all the electrochemical measurements in a three-electrode configuration. The working electrode was a bare electrode or a DNA-modified GCE (2 mm in diameter); a commercial saturated calomel electrode (SCE) and a Pt wire were utilized as reference and counter electrode, respectively. The potentials given in this paper were referred to SCE. The UV spectra were recorded by a Model UV-2102 (UNICO, Shanghai, China) spectrophotometer.

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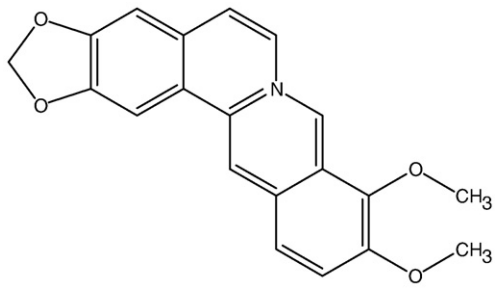

Fig. 1. The chemical structure of berberine.

Solutions of fish test DNA (Shanghai Sangon Company, China) were prepared with doubly distilled water to form 1.0 mg/mL stock solution. The concentration of DNA expressed at a base pair (BP) concentration: [BP]=9.09×10^{-3} M, was determined by the UV absorption at 260 nm using the molar extinction coefficient of 6600 M^{-1} cm^{-1}. A stock solution of 1.0×10^{-3} M berberine (Checkout Institute of Biology Drugs, China) was prepared by dissolving it with doubly distilled water. Octadecylamine (Tianjin Guangfu Fine Chemical Research Institute, China) stock solution was prepared by dissolving it with distilled chloroform and used without further purification. All spreading solutions were kept below and at 5 °C and renewed every 2 weeks. Other chemicals were of analytical reagent grade. High purity nitrogen was used to purge the solution for 10 min prior to each measurement. Each assay was performed at the temperature 298 K (25 °C).

2.2. Procedure

Britton–Robinson (B–R) buffer solution (0.05 M, pH 5.72) was selected as supporting electrolyte. Single-stranded DNA was prepared by heating the double stranded dsDNA solution in water bath at 100 °C for 30 min, then promptly cooling it in ice bath, yielding the so-called denatured ssDNA. All other DNA were double-strand DNA. GC-base pair attached electrodes were used.

2.3. Preparation of DNA-modified electrode

Since DNA as a polyelectrolyte salt is soluble in water, it is not possible to directly use the DNA for preparing LB film. The amphiphilic octadecylamine (ODA) is used to produce LB films. Here, DNA LB film-modified electrodes were prepared from a suspension of DNA in ODA, by dissolving DNA in the sub-phase (water) and spreading the ODA-chloroform solution on the sub-phase surface. When the ODA–DNA suspension was compressed slowly, a monomolecular layer was formed and thus a DNA LB film-modified electrode resulted. For the measurements of the surface pressure (π)–surface area (A) isotherms, a JML-04 LB trough (Shanghai Zhongchen Co., China) was used. The solvent was allowed to evaporate for 30 min before compression. Z-type DNA LB film used in this paper was gained according to a standard vertical dipping procedure by the LB technique.

3. Results and discussion

3.1. Interaction between berberine and DNA in solution

Cyclic voltammetry and differential pulse voltammetry were employed for investigating the interaction between berberine and DNA. Fig. 2 exhibits the cyclic voltammogram of 5.0×10^{-5} M berberine in B–R buffer solution (0.05 M, pH 5.72) at T=298 K (25 °C) within the electrode potential range of $-0.4\le E/V\le-1.25$. Under the selected conditions, a well-defined cathodic current peak was observed. No corresponding anodic peak was observed in the cyclic voltammogram (Fig. 2, Curve A). It indicates that, under these conditions, the electrode reaction of berberine is an irreversible process.

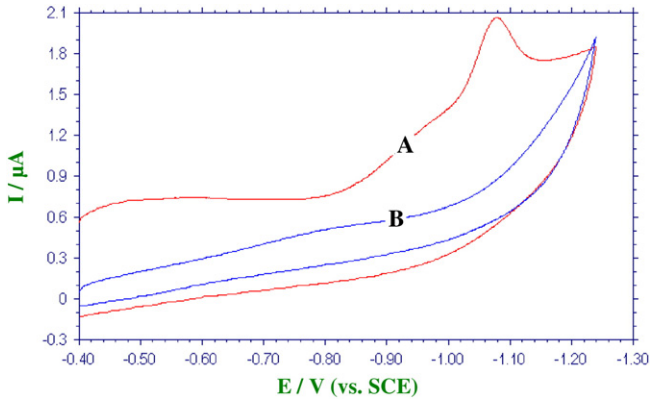

Fig. 2. Cyclic voltammograms of 5.0×10^{-5} M berberine in B–R buffer solution (0.05 M, pH 5.72) (Curve A) and the background (Curve B), with scan rate of v=50 mV/s.

The DPV technique provides the required high current sensitivity and good peak resolution for the investigation of the interaction between berberine and DNA. Prior to carrying out the electrochemical measurements, the mixed solutions of berberine and DNA were stirred for 10 min to reach binding equilibrium. Fig. 3 shows the DPV curves in the presence of 2.0×10^{-5} M berberine (curve A) at different concentrations of DNA (Curves B and C). Under optimum conditions, the peak potential of berberine shifts towards more negative electrode potentials with increasing DNA concentration, consistent with the preponderant electrostatic interaction between (cationic) berberine and the polyanionic DNA [12]. The peak currents decrease with increasing DNA concentration (Fig. 3, Curves B and C), indicating the formation of the DNA–berberine adducts, which may be electro-inactive under this experimental conditions. The peak currents decrease in direct proportion to the DNA concentration, suggesting a means for the analytical determination of DNA concentrations.

When electrostatic interactions are presumed to be dominant in the incorporation process of small molecules carrying positive charges and the polyanionic DNA, the electrochemical interactions are known to be affected by the ionic strength of the solution by ionic screening effects [13]. Here, the DNA-modified electrode was used as the working electrode and prepared by coating the DNA onto the surface of the GCE. The interaction of berberine with DNA was examined in the NaCl concentration range of $2\le$[NaCl]/mM≤80, the NaCl dissolved in B–R buffer solution (0.05 M, pH 5.72). As seen, the peak currents strongly decrease with increasing [NaCl] and nearly disappear when

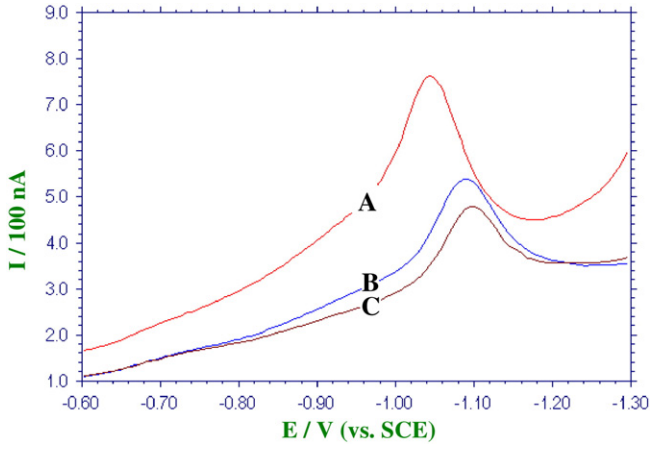

Fig. 3. DPV curves of berberine with different concentrations of DNA at bare GCE. Curve (A): 2.0×10^{-5} M berberine; Curve (B): (A)+1.09×10^{-4} M BP; Curve (C): (A)+1.36×10^{-4} M BP.

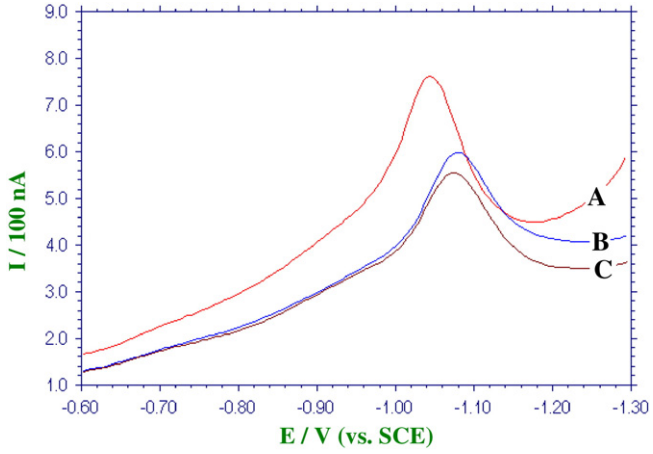

Fig. 4. DPV curves of berberine at bare GCE in B–R buffer solution (pH 5.72). Curve (A): 2.0×10^{-5} M berberine; Curve (B): (A)+9.09×10^{-5} M dsDNA (BP); Curve (C): (A)+9.09×10^{-5} M ssDNA (BP).

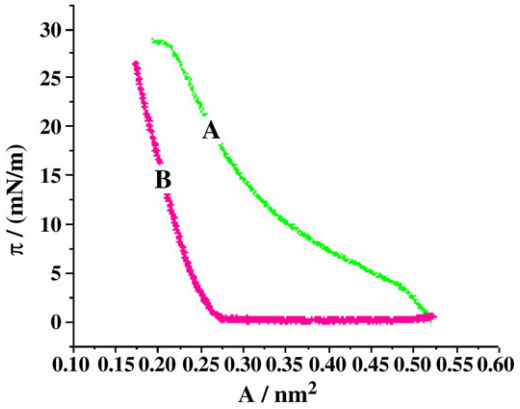

Fig. 5. Surface pressure (π)–area (A) isotherms of ODA monolayers on DNA aqueous solution (Curve A) and on pure water (Curve B).

the ionic strength reaches 6.4 mM. Concomitantly the peak potential of berberine at low ionic strength shifted to more negative values with increasing DNA concentration, confirming the dominance of the electrostatic interactions between berberine and DNA [14]. The shielding effect affects the charge transfer. At low ionic strength, where the ionic shielding is low, the electrochemical signals are expected larger. At high ionic strength, the ionic shielding weakens the electrostatic interactions between berberine and DNA, resulting in the decrease of peak currents, levelling-off at the ionic strength of 1.6 mM.

The peak current decreases with the increasing temperature in the range of $19\le T/°C\le35$. Above 35 °C, the peak currents increase. So far no rationale for the minimum can be offered.

3.2. Comparisons of the interaction of berberine with dsDNA and ssDNA

Fig. 4 shows the differential pulse voltammetry responses of 2.0×10^{-5} M berberine (Fig. 4A) and 9.09×10^{-5} M dsDNA (BP) (Fig. 4B) as well as 9.09×10^{-5} M ssDNA (BP) (Fig. 4C), respectively. The peak currents decrease and the peak potentials shift to more negative electrode potentials for both dsDNA and ssDNA. Moreover the peak current obtained with dsDNA is slightly higher than that obtained with ssDNA. This result indicates that berberine interacts with ssDNA more strongly. The decrease in the currents with ssDNA is either due to an increase in the electrostatic binding of berberine to ssDNA or due to a decrease in the electron transfer of berberine through the ssDNA, or probably both.

3.3. Interaction of berberine with DNA modified at the GCE

3.3.1. The π/A-isotherms of octadecylamine and octadecylamine-DNA LB films

Fig. 5 shows the surface pressure (π) versus area (A) isotherm of octadecylamine (ODA) at the pure water–air interface and at the aqueous DNA solution–air interface, respectively. The difference for ODA is due to the different sub-phase. For DNA/ODA composite Langmuir monolayer (Fig. 5A), a rapid expansion of the molecule area is found due to the decrease of surface pressure. The limiting area value is $A_{(lim)}$=0.38 nm^2 and the collapsing pressure is $\pi_{(coll)}$=27.3 mN/m. Compared with the ODA monolayer (Fig. 5B) there is a steep rise in surface pressure and the limiting area is 0.24 nm^2 per molecule, suggesting the formation of a DNA/ODA composite film. The migration of the DNA molecules from sub-phase into ODA Langmuir monolayer is dominantly driven by electrostatic interactions between the negatively charged DNA and the cationic ODA molecules [15]. It is known that DNA/ODA conjugate monolayers at the air–water interface are stable and behave like ideal amphiphilic complexes [16]. The LB technique is confirmed to be suited to immobilize DNA onto the electrode surface and the DNA retains its native form as a double-helical structure.

To provide further information about the structure and the electrochemical properties of DNA Langmuir films, redox couples of Fe$(CN)_6^{3-/4-}$ were used as electrochemical probes. The cyclic voltammograms were recorded for 5.0×10^{-4} M $K_3Fe(CN)_6$ at the bare GCE and the DNA-modified GCE. The peak currents decreased and the peak potentials shifted slightly at the DNA-modified GCE relative to the bare GCE. This means that Fe$(CN)_6^{3-/4-}$ can pass through the DNA/ODA film and reach the GCE surface for exchanging electrons, despite some electrostatic repulsion between DNA and Fe$(CN)_6^{3-/4-}$, indicating that DNA had been immobilized onto the electrode surface by the Langmuir technique.

3.3.2. Interaction of berberine with DNA-modified electrode

Fig. 6 shows the DPV curves of 1.5×10^{-5} M berberine at the bare electrode (Curve A) and the DNA-modified electrode (Curve B) with a scan potential range of $-0.6\le E/V\le-1.35$. Berberine is reduced at both electrodes, but the peak current decreases and peak potential shifts to more negative values at the DNA-modified electrode in comparison with the bare electrode. The peak current at the bare electrode is seven times larger than that at the DNA-GCE, consistent with the strong electrostatic binding of berberine to DNA at the DNA-GCE [17].

The data correlation of the peak currents i_p and the scan rate v is linear in the scan range of $30\le v/(mV/s)\le200$, which is characteristic of thin layers [18]. The results suggest that the anticancer drug berberine

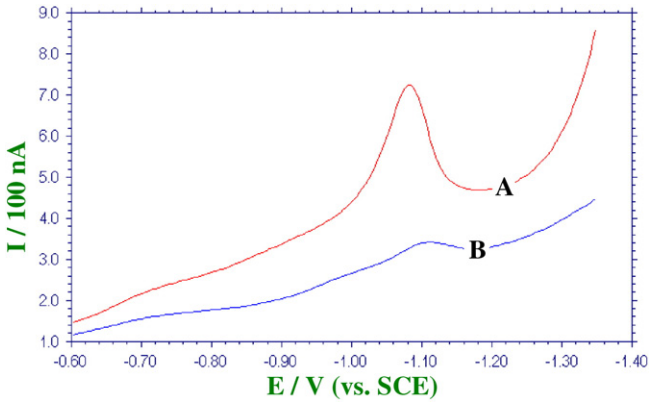

Fig. 6. DPV curves of 1.5×10^{-5} M berberine at bare electrode (Curve A) and DNA-modified electrode (Curve B) in B–R buffer solution (pH 5.72).

associates with immobilized DNA and thus the electrode process is adsorption controlled. The open circuit DPV effect on the accumulation time indicates that the peak current continuously increases within 6 min and saturates at longer accumulation time. As a compromise between the sensitivity and the working efficiency, the optimal accumulation time of 6 min was selected for studying the interaction of berberine with DNA immobilized at GCE.

3.4. UV spectroscopy for characterization of the interaction of Berberine with DNA

As known, DNA in aqueous solution exhibits an absorbance peak at 260 nm (Fig. 7A). The berberine exhibits absorbance peaks at 227 nm, 262 nm, 344 nm, respectively (Fig. 7B), and a broad low absorption in the range of 380≤λ/nm≤460. The absorbances at 262 nm and 344 nm correspond to the π-π^* transitions of the isoquinolin structure, and the band centered at about 424 nm arises from the n-π^* transition [19]. The absorption spectra change and the appearance of isobestic wavelengths indicate interactions with DNA (Fig. 7, Curves C and D). Hypochromic effects are particularly pronounced at 260 nm. The isobestic point at about 358 nm reflects the virtual overlap of the π electron cloud of the drug berberine indicating that the intercalative binding mode of berberine with DNA at this part is much weaker [20]. The results also indicate that the DNA retains its double-helical structure in the mixed solution, confirming the dominance of electrostatic interactions.

3.5. Association equilibrium constant and stoichiometric coefficient of $DNA{\cdot}BE_m$

It is known that berberine (BE) [21] and DNA base pairs associate to one type of cooperative complex $DNA{\cdot}BE_m$ according to the reaction scheme:

$$\mathrm{DNA} + m\mathrm{BE} = \mathrm{DNA}\cdot\mathrm{BE}_m \tag{1}$$

In terms of the overall Hill cooperativity model, the fraction of berberine bound to DNA is given by:

$$f = [\mathrm{DNA}\cdot\mathrm{BE}_m]/[\mathrm{DNA}\cdot\mathrm{BE}_m]_{\max} = [\mathrm{BE}]^m/([\mathrm{BE}]^m + K_\mathrm{d}^m) \tag{2}$$

and

$$K_\mathrm{d}^m = [\mathrm{BE}]^m(1-f)/f \tag{3}$$

where $[DNA{\cdot}BE_m]_{max}$ is the maximum concentration of complexed base pairs, [BE] the concentration of free berberine. K_d is the dissociation equilibrium constant and m is the Hill coefficient.

Note that $K_d = [BE]_{0.5}$ at $f = 0.5$, i.e., at half occupation. The association constant K_a is given by the reciprocity: $K_a = K_d^{-1}$. It is not advisable to use the overall constant $K = (K_a)^m$, which would have the physically meaningless dimension of M^{-m}.

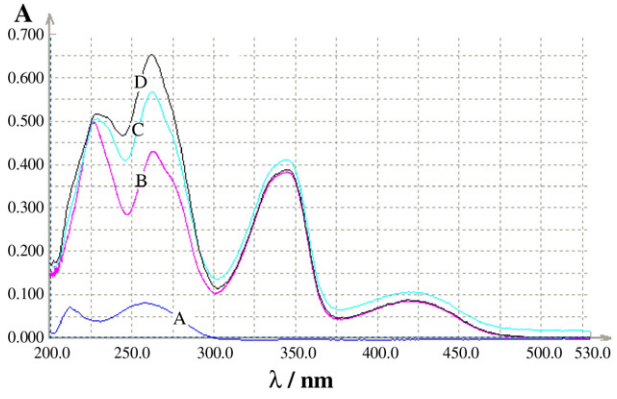

Fig. 7. The UV spectra of berberine and $DNA{\cdot}BE_m$ complex in B–R buffer solution (0.05 M, pH 5.72). Curve (A): 3.64×10^{-5} M BP; Curve (B): 1.4×10^{-5} M berberine; Curve (C): (B)+5.43×10^{-5} M BP; Curve (D): (B)+1.10×10^{-4} M BP.

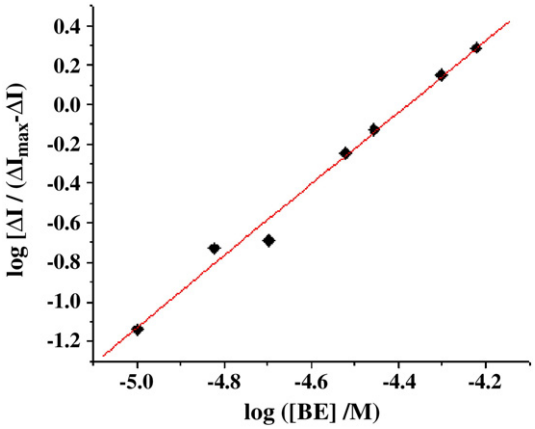

Fig. 8. The relationship between $\Delta I/(\Delta I_{max}-I)$ and $[BE]/M$ on double-logarithmic scale at the GCE in the B–R buffer solution (0.05 M, pH 5.72) in the absence and presence of DNA, respectively.

The interaction partners for BE are the base pairs of DNA. Mass conservation dictates that the concentration of free DNA base pairs, available for binding of berberine is given by:

$$[\mathrm{DNA}] = [\mathrm{DNA}\cdot\mathrm{BE}_m]_{\max} - [\mathrm{DNA}\cdot\mathrm{BE}_m] \tag{4}$$

Mass conservation for berberine dictates that:

$$[\mathrm{BE}] = [\mathrm{BE}]_\mathrm{o} - m[\mathrm{DNA}\cdot\mathrm{BE}_m] \tag{5}$$

where $[BE]_o$ is the total concentration of berberine. It is found that under the given experimental condition the current I is given by:

$$I = k\cdot[\mathrm{BE}] \tag{6}$$

where k is a constant. In line with this relationship the current difference ΔI is defined as:

$$\Delta I = I(\mathrm{BE}_0) - I(\mathrm{BE}) \tag{7}$$

Insertion of Eqs. (5) and (6) into Eq. (7) yields:

$$\Delta I = k([\mathrm{BE}_\mathrm{o}] - [\mathrm{BE}]) = k\cdot m\cdot[\mathrm{DNA}\cdot\mathrm{BE}_m] \tag{8}$$

$$\Delta I_{\max} = k\cdot m\cdot[\mathrm{DNA}\cdot\mathrm{BE}_m]_{\max} \tag{9}$$

From Eq. (3), we obtain that:

$$\log[f/(1-f)] = m\log(K_\mathrm{a}/M) + m\log([\mathrm{BE}]/M) \tag{10}$$

Insertion of Eqs. (8) and (9) into Eq. (10) yields:

$$\log\left[\frac{\Delta I}{\Delta I_{\max} - \Delta I}\right] = m\log(K_\mathrm{a}/M) + m\log([\mathrm{BE}]/M) \tag{11}$$

If DNA and berberine form a single adduct, the plot $\log[\Delta I/(\Delta I_{max}-\Delta I)]$ versus $\log([BE]/M)$ is linear with a slope of m. Data fit (Fig. 8) yields $m = 1.82$ and $K_a = 2.2(\pm0.2)\times10^4\ M^{-1}$, thus $K_d = 4.6(\pm0.3)\times10^{-5}$ M, which is

Table 1
The analytical data for sample

Sample	Added (10^{-5} M)	Found (10^{-5} M)	Recovery (%)	RSD (%)	Results from UV (10^{-5} M)
DNA (BP)	5.45	5.19	95.23	3.11	5.21
DNA (BP)	9.09	8.79	96.70	2.94	9.19
DNA (BP)	13.63	13.93	102.20	2.81	13.45

in the concentration range of the applied berberine. The stoichiometry of the cooperative berberine binding is thus at least 2 per base pair unit.

3.6. Analytical application

Under the optimized conditions mentioned above, the experimental data for the calibration plot were recorded by DPV. The peak currents of berberine with different concentration of DNA were then used to establish the calibration curve. The decrease of peak current of 3.0×10^{-5} M berberine was proportionate to the concentration of DNA (BP) from 1.82×10^{-5} M to 1.64×10^{-4} M with the detection limit of 1.45×10^{-5} M. The linear regression equation is Δi_p (10^{-7} A)=3.70+0.68 C_{BP} (10^{-5} M) with a correlation coefficient γ=0.995.

In order to examine the applicability for the proposed method, the standard addition method was employed to determine the recovery of DNA with the addition of fish test DNA and the relative standard deviation, respectively. The standard solution was added into the sample solution, regarding the mixed solution including familiar ions (K^+, Na^+, Cl^- and SO_4^{2-}) as a suitable synthetic sample solution. The analysis data for the synthetic sample solution is given in Table 1.

4. Conclusions

The interaction of anticancer herbal drug berberine with DNA can be quantified in terms of the Hill model of cooperative interactions. The results serve as a reference for the study of berberine with DNA base pairs in the natural environment of living cells.

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