Colloids and Surfaces B: Biointerfaces 82 (2011) 438–442

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Interaction of an antidepressant buzepide methiodide with DNA immobilized on the glassy carbon electrode

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ARTICLE INFO

Article history:
Received 18 August 2010
Received in revised form
20 September 2010
Accepted 20 September 2010
Available online 29 September 2010

Keywords:
Buzepide methiodide
DNA interaction
DNA modified electrode
Voltammetric and spectroscopic approach
Intercalative binding

ABSTRACT

In the present study, a DNA-biosensor was prepared using immobilization technique to investigate the interaction between an antidepressant, buzepide methiodide (BZP) and calf thymus DNA. BZP showed a quasireversible peak in Britton–Robinson (BR) buffer of pH 5 at bare glassy carbon electrode (GCE). At DNA modified GCE, the peak potential of BZP was observed to be shifted towards positive potential revealing intercalative mode of binding. The binding constant and stoichiometry between DNA and BZP are calculated to be $1.908 \times 10^5\ M^{-1}$ and 0.982, respectively. The spectroscopic techniques viz., spectrofluorescence and UV–vis absorption have also been employed to understand the interaction between BZP and DNA. The results serve as a reference for the interaction of BZP with DNA base pairs in the natural environment of living cells.

1. Introduction

The binding nature and dynamics of small molecules to DNA is an important fundamental issue in life science and is useful in understanding the structure and function of DNA [1]. DNA binding studies play an important role in life phenomena such as mutations of genetic information leading to diseases by causing changes in replication and transcription of DNA. A precise understanding of their DNA binding affinity and the cleavage mechanism would provide insight into the nature and structure of the reactive intermediate and would be potentially useful in the design of new site-specific scission reagents and chemical probes [2].

Electrochemical techniques have attracted appreciable attention due to the inherent specificity and sensitivity. Recently, these techniques are being employed to investigate the interaction mechanisms and dynamics of the interactions between DNA and drugs [3,4]. By observing the electrochemical signal related to DNA–drug interactions, it is possible to propose the mechanism of interaction, conformation of adduct, nature of the complex formed and to evaluate the binding constant. Electrochemical investigations of small molecule–DNA interactions provide useful complement to the results obtained by spectroscopic methods.

Recently, there has been considerable interest in developing DNA electrochemical biosensors for rapid and inexpensive diagnosis of genetic diseases and for other applications [5,6]. Electrochemical DNA biosensors comprise a nucleic acid recognition layer that is immobilized over an electrochemical transducer. The role of the nucleic acid recognition layer is to detect the changes that occurred in the DNA structure during interaction with DNA-binding molecules. The pre- and post-electrochemical signals of DNA–drug system provides good evidence for the mechanism of interaction [7].

BZP is an antidepressant [8] and chemically known as 1-(4-amino-4-oxo-3,3-diphenylbutyl)hexahydro-1-methyl-1Hazepinium iodide. It is used in the treatment of functional intestinal disorders in combination with haloperidol [9]. In a metaanalysis of Randomized Clinical Trial (RCT), it was concluded that the antidepressants might be effective in reducing Irritable Bowel Syndrome (IBS) symptoms in about one-third of patients [10]. Since BZP is an antidepressant, attempts have been made to assess its effect to treat the IBS [11].

Attempts have not been made till date to investigate the interaction of BZP with calf thymus DNA. The aim of the present work is to study the interaction between BZP and DNA in solution at bare GCE and at the surface of GCE using DNA biosensor.

2. Materials and methods

2.1. Apparatus

Electrochemical studies were carried out on a CHI-1110a electrochemical Analyzer (CH Instruments Ltd. Co., USA, version 4.01)

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doi:10.1016/j.colsurfb.2010.09.017

electrode system, consisting of GCE (3 mm diameter) as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl (3 M KCl) as reference electrode. Fluorescence measurements were performed on a Hitachi spectrofluorimeter Model F-7000 (Tokyo, Japan) equipped with a 150 W Xenon lamp and a slit width of 5 nm. The UV-absorption spectra were recorded on a double beam CARY 50-BIO UV–visible spectrophotometer (Varian, Australia) with 1 cm matched quartz cells.

2.2. Reagents

Double-stranded calf thymus DNA (dsDNA) was purchased from Sigma. The dsDNA was dissolved in water and stored at 4 °C. The concentration of DNA was determined according to absorbance at 260 nm after establishing the absorbance ratio, A_{260}/A_{280} to be in the range of 1.80–1.90. This indicated that the DNA was free from protein. The molar extinction coefficient, ε_{260} was taken as 6600 L mol^{-1} cm^{-1}. Denatured single-stranded DNA (ssDNA) was produced by heating the dsDNA solution in a water bath at 97 °C for 5 min, immediately followed by rapid cooling in an ice bath. A stock solution of 0.25 mM ethidium bromide (EB) was prepared in water. A stock solution of BZP (2.5 mM) was prepared in acetonitrile–water (20:80, v/v) and stored in a refrigerator at 4 °C. Standard solutions were prepared by diluting the stock solution with a selected supporting electrolyte. The chemicals used were of Analar grade. All the solutions were prepared in milli-pore water.

2.3. Electrode modification

DNA modified GCE was prepared by following the procedure reported earlier [12]. Before immobilization, GCE was polished with 0.05 μm α-alumina powder on a polishing micro-cloth until a mirror-like surface was obtained. It was washed with absolute alcohol and milli-pore water in an ultrasonic bath to remove the adsorbents on the electrode. For immobilization of DNA on GCE, the electrode was inverted upside and 5 μl of 10 mM ct DNA [12,13] solution was dropped on the electrode tip with the help of a micropipette and allowed the electrode to dry. It was then rinsed with millipore water and supporting electrolyte to remove the unadsorbed DNA. This is denoted as GCE–DNA throughout.

2.4. Voltammetric studies on interactions of BZP with DNA

For binding studies, differential pulse voltammogram of 5×10^{-5} M BZP (fixed concentration) was recorded in presence of increasing amounts of DNA in BR buffer of pH 5. For each addition of DNA to BZP, an interaction time of 10 min was maintained and differential pulse voltammogram was recorded.

2.5. UV–vis absorption measurements

The UV–vis absorption measurements of BZP (5×10^{-5} M) in the absence and presence of increasing concentrations dsDNA/ssDNA (0–250 μM) were made in the range of 230–330 nm.

2.6. Fluorescence measurements

The fluorescence spectra of BZP in presence and absence of DNA were recorded upon excitation of BZP at 230 nm. For this, the concentration of BZP was fixed at 5×10^{-5} M while that of DNA was varied form 0 to 300 μM.

The fluorescence spectra of EB in presence and absence of DNA were recorded in BR buffer at 595 nm upon excitation at 525 nm. In the second step, emission spectra of EB-DNA were also recorded in presence of increasing amounts of BZP.

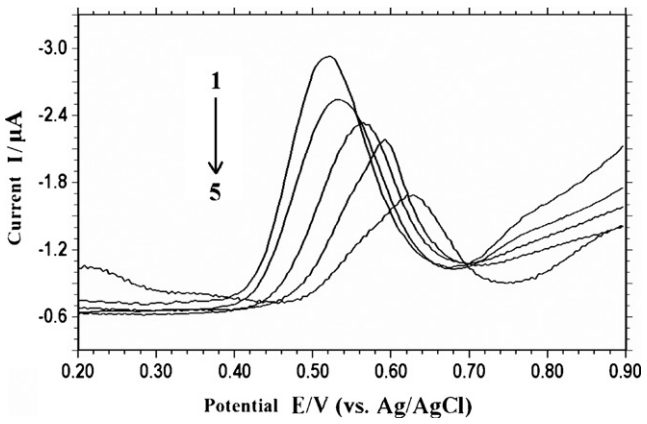

Fig. 1. Differential pulse voltammograms of (1) 50 μM BZP in the absence and presence of (2) 50, (3) 100, (4) 150, and (5) 200 μM DNA in BR buffer of pH 5.

3. Results and discussion

3.1. Electrochemical behavior of BZP

In our previous work [14], we have reported electrochemical behavior of BZP at GCE. BZP exhibited a pair of quasireversible peaks viz., the oxidation peak potential at 0.573 V and reduction peak potential at 0.483 V in BR buffer of pH 5 (figure not shown). The peaks were observed to be diffusion controlled in the scan rate range of 5–500 mV s^{-1} and pH dependent.

3.2. Interaction of BZP with DNA

Differential pulse voltammetric technique provides higher sensitivity and better peak resolution compared to cyclic voltammetry for studying the electrochemical behavior of biological systems [15]. Therefore, we have employed differential pulse voltammetric technique to investigate the interaction of BZP with DNA. Considering the sensitivity, we chose the anodic peak (peak 1) as probe to study the interaction between BZP and DNA. The differential pulse voltammogram of 50 μM BZP in presence and absence of DNA at GCE in BR buffer (pH 5) were recorded (Fig. 1).

Upon the addition of DNA to BZP solution, marked decrease in oxidation peak current of BZP with a large positive shift in peak potential was noticed. Further, no new oxidation peaks were noticed. This revealed the interaction between DNA and BZP. The interaction of BZP was found to depend on time. In order to find out the interaction time, we recorded the differential pulse voltammogram of BZP in presence of DNA at different time intervals. We observed a significant decrease in peak current of BZP up to 10 min. After 10 min, the peak current of BZP remained almost constant. Therefore, an interaction time of 10 min was maintained throughout after the addition of DNA. The electrode was washed thoroughly after each measurement.

The decrease in peak current of BZP upon the addition of DNA may be attributed to different mechanisms as explained below: The changes in electrochemical kinetics of BZP in presence of DNA [16] may decrease the peak current. The major electrochemical kinetic parameters (α and ko) of BZP in presence and absence of DNA can demonstrate whether DNA influences the electrochemical kinetics of BZP or not. The values of α and ko were evaluated to be 0.55 and 1.08 s^{-1} in the absence of DNA and 0.56 and 1.10 s^{-1} in presence of DNA. Since, the values of α and ko in the absence and presence of DNA are almost same, we propose that the DNA did not alter the electrochemical kinetics of oxidation of BZP.

In order to prove that the decrease in peak current is not due to increase in viscosity of the solution or the blockage of the elec-

trode surface by DNA adsorption [17], a special cyclic voltammetric experiment was designed in $K_4[Fe(CN)_6]$ solution [18] in presence and absence of DNA. The $[Fe(CN)_6]^{4-}$ ions did not interact with DNA due to coulombic repulsion between their negative charges. The peak currents of $[Fe(CN)_6]^{4-}$ were observed to be almost same in presence and absence of DNA. Hence, we conclude that the decrease in peak current may not be due to change in viscosity of the solution or DNA adsorption.

Another possibility for decrease in peak current may be due to the competitive adsorption between BZP and DNA. Since, the electrode process of BZP is diffusion controlled [14] and since no adsorption time is given in our experiments, we rule out the possibility of competitive adsorption between BZP and DNA. It is reported [19] that under the conditions of lower concentration species, larger electrode area and shorter accumulation time, competitive adsorption between a ligand and DNA does not exist. In view of the above, the decrease in peak current of BZP upon the addition of DNA was mainly attributed to decrease in equilibrium concentration of free BZP. This decrease in the concentration of BZP was attributed to the formation of an electrochemically inactive BZP-DNA system that did not undergo oxidation at GCE. This indicated that the BZP has interacted with DNA. Bard et al. [20] have reported the positive shift in peak potential for hydrophobic interactions (for intercalators) and negative shift for electrostatic interactions. The positive shift in peak potential observed in the present work indicated the presence of intercalative mode of binding between DNA and BZP.

If it is assumed that the interaction of DNA with BZP produces only a single complex, DNA–BZPm, then the equation shown below holds good [21,22]:

$$\mathrm{DNA} + m\mathrm{BZP} = \mathrm{DNA} - \mathrm{BZP}m \tag{1}$$

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

$$f = \frac{[\mathrm{DNA} - \mathrm{BZP}_m]}{[\mathrm{DNA} - \mathrm{BZP}_m]_{\max}} = \frac{[\mathrm{BZP}]^m}{([\mathrm{BZP}]^m + K_\mathrm{d}^m)} \tag{2}$$

where $[\mathrm{DNA} - \mathrm{BZP}_m]_{\max}$ represents the maximum concentration of complexed DNA, and [BZP] is the concentration of free BZP. K_d is the equilibrium dissociation constant and m is the Hill coefficient. Then, the following formula can be deduced:

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

Note that $K_\mathrm{d} = [\mathrm{BZP}]_{0.5}$ at $f = 0.5$, i.e., at half occupation. The binding constant, K_a is given by the reciprocal of K_d, i.e., $K_\mathrm{a} = K_\mathrm{d}^{-1}$.

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

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

For the same reason

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

where $[\mathrm{BZP}]_\mathrm{o}$ is the total concentration of BZP. Under the given experimental conditions, the current I is given as,

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

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

$$\Delta I = I(\mathrm{BZP})_\mathrm{o} - I(\mathrm{BZP}) \tag{7}$$

Substituting the values of Eqs. (5) and (6) in Eq. (7) we get

$$\Delta I = k([\mathrm{BZP}]_\mathrm{o} - [\mathrm{BZP}] = km[\mathrm{DNA} - \mathrm{BZP}_m] \tag{8}$$

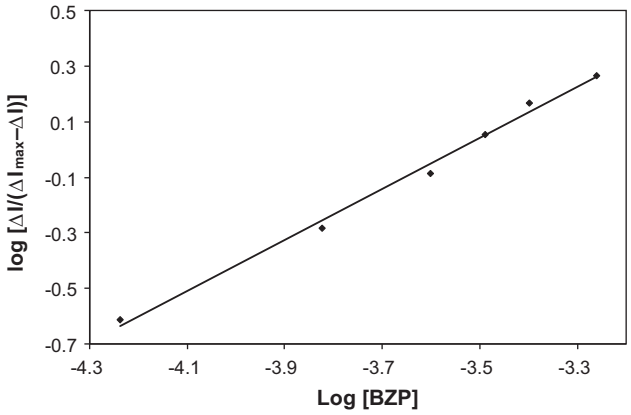

Fig. 2. The plot of $\log[\Delta I/(\Delta I_{\max} - \Delta I)]$ versus log[BZP].

$$\Delta I_{\max} = km([\mathrm{DNA} - \mathrm{BZP}_m]_{\max} \tag{9}$$

From Eq. (3), we obtain the following equation:

$$\log\left[\frac{f}{1-f}\right] = m\ \log K_\mathrm{a} + m\ \log[\mathrm{BZP}] \tag{10}$$

Substituting the values of Eqs. (8) and (9) in Eq. (10) we obtain

$$\log\left[\frac{\Delta I}{\Delta I_{\max} - \Delta I}\right] = m\ \log K_\mathrm{a} + m\ \log[\mathrm{BZP}] \tag{11}$$

If DNA interacts with BZP to form a single adduct, then the plot of $\log [\Delta I/(\Delta I_{\max} - \Delta I)]$ versus log [BZP] is expected to be linear with the slope of m. The experimental data fit (Fig. 2) yielded the values of m and K_a to be 0.982 and $1.908 \times 10^5\ \mathrm{M}^{-1}$, respectively. The stoichiometry of the cooperative BZP binding was at least 1 per base pair unit. Thus, the formation of a stable 1:1 complex of DNA–BZP was proposed.

3.3. Comparison of the interaction of BZP-dsDNA with BZP ssDNA

Three modes of binding proposed for the interaction between small molecules and DNA double helix include intercalative binding, groove binding and electrostatic binding [23,24]. Among these, intercalation and groove binding modes are dependent on DNA double helix. But, the electrostatic binding occurs out of the groove of the DNA. If the interaction models are intercalative and groove binding, the interaction capability would decrease in presence of denatured DNA. However, the electrostatic interactions may continue to operate even after DNA denaturation. The differential pulse voltammogram of BZP in presence and absence of ssDNA and dsDNA are shown in Fig. 3.

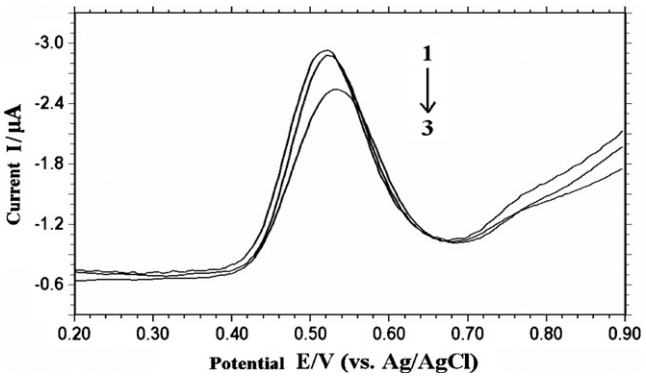

Fig. 3. Differential pulse voltammograms of (1) 50 μM BZP in the absence and presence of (2) 50 μM ss DNA and (3) 50 μM ds DNA in BR buffer of pH 5.

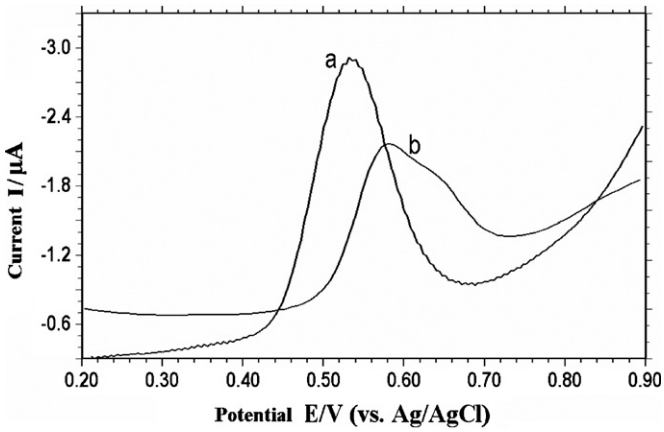

Fig. 4. Cyclic voltammograms of 50 μM BZP at GCE (a) and GC-DNA (b) electrodes.

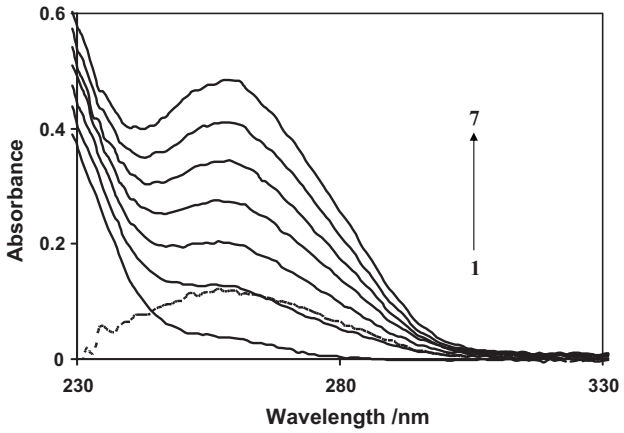

Fig. 5. UV–vis absorption spectra of (1) 50 μM BZP in the presence of increasing concentration of DNA (2) 50, (3) 100, (4) 150, (5) 200, (6) 250 and (7) 300 μM DNA. Dotted line indicates 50 μM DNA only.

No appreciable changes in differential pulse voltammogram of BZP were observed upon the addition of ssDNA as ssDNA did not possess double helix. This ruled out the interaction between BZP and ssDNA. This result further supported that the BZP intercalated into double helix of DNA.

3.4. Interaction of BZP with DNA at electrode surface

DNA-GCE electrode was prepared using immobilization technique to study the interaction between BZP and DNA at the electrode surface. The immobilization step of the capture probe could lead to a well-defined probe orientation, which allows for ready accessibility of the target. Thus, specific DNA probe–target interactions and charge transfer reactions give electrical signals directly which can be easily monitored [6].

From the cyclic voltammogram of 1.0×10^{-4} M $K_3[Fe(CN)_6]$ at the bare electrode and the DNA-GCE, we found that the peak currents of $K_3[Fe(CN)_6]$ decreased significantly at the DNA modified electrode. This was attributed to the repulsion between the negatively charged DNA molecules and $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ ions. These results indicated that the DNA was immobilized at the electrode surface [12].

Fig. 4 shows the differential pulse voltammograms of 5×10^{-5} M BZP at the bare and DNA-GCE. From the figure, it was evident that the BZP was oxidized at both the electrodes. The peak current of BZP was noticed to be decreased with positive shift in peak potential at DNA-GCE. This decrease in peak current could be attributed to the ordered surface structure of DNA on the surface of the electrode, making BZP less facile to reach the electrode surface and for the electron transfer [25]. We proposed the intercalative mode of interaction between BZP and DNA based on the observation of positive shift in peak potential at DNA-GCE [26]. Further, the effect of interaction time on the binding of BZP to DNA at DNA-GCE was investigated. The results indicated that the peak currents increased up to 1 h and became almost constant at longer accumulation time intervals at DNA-GCE. Therefore, an optimal accumulation time of 1 h was maintained for studying the interaction of BZP with DNA at DNA-GCE.

3.5. UV–vis absorption studies

The absorption spectrum of BZP showed an absorption maximum at 254 nm (Fig. 5) in BR buffer of pH 5.0. The absorbance values of BZP at 254 nm increased regularly with increase in the concentration of DNA with a red shift of 5 nm. This indicated the interaction between BZP and DNA through intercalation [19].

Based on the variations in the absorption spectra of BZP in presence of DNA, the binding constant, K was calculated using the following equation [27]:

$$\frac{A_0}{(A - A_0)} = \left[\frac{\varepsilon_G}{\varepsilon_{H\text{-}G} - \varepsilon_G}\right] + \frac{\varepsilon_G}{\{(\varepsilon_{H\text{-}G} - \varepsilon_G)K[DNA]\}}$$

where A_0 and A are the absorbances of drug in the absence and presence of DNA, ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of drug and its complex with DNA, respectively. The plot of $A_0/(A - A_0)$ versus 1/[DNA] was constructed using the data from the absorption titration. A linear fitting of the data yielded the K value to be 1.788×10^5 M^{-1}. This value was noticed to be close to that obtained from the electrochemical data (1.908×10^5 M^{-1}).

3.6. Fluorescence studies

The binding of BZP to DNA was further studied by fluorescence spectroscopy. BZP exhibited an emission maximum at 325 nm upon excitation at 230 nm. The fluorescence intensity of BZP decreased in presence of increasing amounts of DNA indicating the interaction between BZP and DNA (figure not shown).

In order to understand the mode of binding between BZP and DNA, we have employed ethidium bromide (EB), a well known interacalotor. EB has a planar structure that binds DNA through intercalation mode [28,29]. The fluorescence intensity of EB increases upon DNA intercalation. If BZP intercalates into DNA, it decreases the binding sites in DNA available for EB. This results

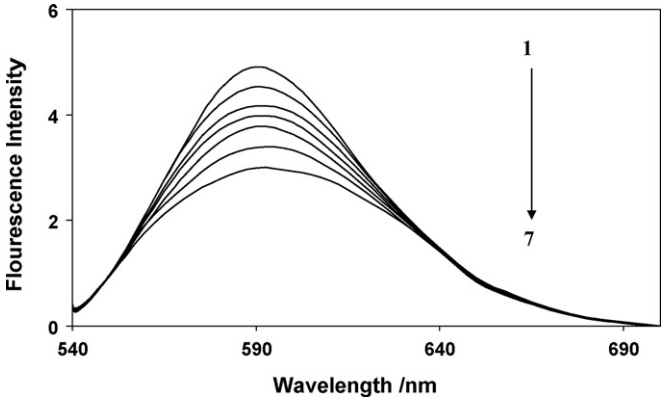

Fig. 6. Fluorescence emission spectra of 1.25 μM EB bound to 70 μM DNA (1), and quenching of EB DNA by BZP. The BZP concentrations were (1) 0, (2) 40, (3) 80, (4) 120, (5) 160, (6) 200 and (7) 240 μM.

in the decrease of fluorescence intensity of EB–DNA system. Fig. 6 shows the fluorescence emission spectra of EB with and without DNA and the changes in fluorescence intensity of EB–DNA system upon the addition of BZP.

It is evident from the observation that the EB was displaced by BZP thereby indicating the presence of intercalative mode of binding between BZP and DNA.

4. Conclusions

In this work, the interaction of BZP with DNA is investigated by electrochemical and spectroscopic methods. The binding of BZP to DNA results in a series of changes in electrochemical behavior and spectral characteristics. These results revealed the presence of intercalative mode of interaction between BZP and DNA. The present study demonstrated that the electrochemical method combined with spectroscopic methods provide significant promise to study the mechanism of interaction of DNA with targeting compounds which in turn useful to design DNA targeted compounds.

Acknowledgements

We are grateful to the University Grants Commission, New Delhi, for financial assistance [F. No. 34/374/2008(SR) dated 30-12-2008). Thanks are also due to the authorities of the Karnatak University, Dharwad, for providing the necessary facilities.

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