Journal of Saudi Chemical Society (2016) **20**, S236–S240

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Voltammetric interaction of vitamin B_1 with DNA at multiwalled carbon nanotube paste electrode

Pradeep Kumar Brahman *, Riyaz Ahmad Dar, Krishna Sadashiv Pitre

Chemical Technology Laboratory, Dr. H.S. Gour University (A Central University), Sagar 470 003, M.P., India

Received 23 July 2012; accepted 22 September 2012
Available online 15 November 2012

KEYWORDS

Vitamin B_1;
DPV;
MWCNTPE

Abstract The interaction between vitamin B_1 and DNA was studied with differential pulse voltammetry (DPV) at the bare and DNA modified MWCNTPE. The differential pulse voltammograms of vitamin B_1 showed that peak potentials shifted to a more negative value and peak currents decreased with the addition of DNA, indicating the dominance of electrostatic interactions. The combining constant (β) and combining number (m) of DNA–mVB_1 were determined too. The work has been supplemented by an UV spectral study.

1. Introduction

Vitamins are an essential group of food ingredients which have to be supplied in sufficient amounts with diet. Vitamins are a broad group of organic compounds that are minor, but essential constituents of food required for normal growth, self maintenance and functioning of human and animal bodies (Poongothai et al., 2010). Vitamin B_1 (Scheme 1) contains pyrimidine and a thiazole ring. It performs important biochemical functions as a coenzyme thiamine pyrophosphate (TPP) which is involved in energy metabolism. Vitamin B_1 (VB_1) is one of the eight vitamins that make up the powerful group called the vitamin B complex. It plays a major role in the good health of the body as well as in sound mental health (Akyilmaz et al., 2006; Zhang et al., 2010; Markopoulou et al., 2002).

The investigation of DNA interactions (Lu et al., 2000; Radi, 2010; Wang et al., 2003; Dogan-Topal and Ozkan, 2011; Liping et al., 2006; Raufa et al., 2005; Yaheng et al., 2008; Nawaz et al., 2006; Brahman et al., 2012) with small molecules is an important fundamental issue on life science, which is of great importance to understand the action mechanisms of some anti-tumour and anti-viral drugs. The interaction of DNA with small molecules can cause the change of conformation as well as the charge distribution state of DNA. Carbon nano tube paste electrodes (CNTPE) have acquired greater importance in the field of electrochemistry due to their low residual current and less noise and because they are very economic and easy to prepare and replace.

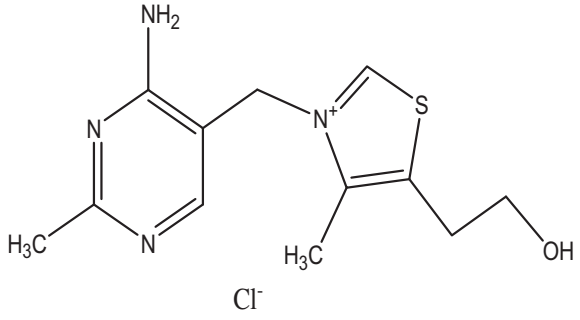
In this work, electrochemical data are reported on the interaction between vitamin B_1 and DNA, obtained by applying cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at multiwalled carbon nanotube paste electrode. The results suggest that the principal interactions between vitamin B_1 and DNA are cooperative and dominant of electrostatic nature.

* Corresponding author.
E-mail address: tiwari25_pradeep@yahoo.com (P.K. Brahman).

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2012.09.021



Scheme 1 Chemical structure of vitamin B1.

2. Experimental

2.1. Chemicals and reagents

All reagents were of AR grade purchased commercially. Calf thymus DNA and vitamin B_1 of Himedia Ltd. Mumbai were used. Solutions were prepared using double distilled water. Stock standard solution of vitamin B_1 was prepared in double distilled water.

2.2. Apparatus

All voltammetric experiments were performed with a Ω Metrohm model 797 VA Computrace (ion analyzer, Switzerland.) through electrochemical software version 3.1. A three-electrode cell was employed incorporating a working MWCNTPE, an Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode. A Systronics digital μpH meter model-361 was used for pH measurements. All experiments were performed at room temperature and the dissolved oxygen was removed by passing pure nitrogen through the solutions. A double beam UV–vis spectrophotometer (Elico-SL-164) was used to measure absorbance of vitamin B1 solutions.

2.3. Preparation of multiwalled carbon nanotube paste electrode

The MWCNTPE was prepared by mixing MWCNT powder, graphite powder and high viscosity paraffin (density = 0.88 g cm^{-3}) from Sigma Aldrich in a ratio of 10:60:30% (w/w) in a mortar. A portion of the resulting paste was then placed in the bottom of a Teflon well. The electrical connection was implemented by a copper wire lead fitted into the Teflon well. The surface of the resulting paste electrodes was smoothened on a weighing paper and rinsed carefully with distilled water. The polished electrode was pretreated at the optimized potential of + 1.80 V vs. Ag/AgCl for 5 min for electrochemical activation of electrode surface. Pretreatment was carried out in 0.2 M acetate buffer solution (pH 4.80) containing 20 mM of NaCl without stirring.

2.4. Fabrication of ds-DNA modified MWCNTPE

MWCNTPE was polished to a mirror-like surface with 1.0, 0.3 and 0.05 μm alumina slurry on micro cloth pads, and sonicated in ethanol and water. The freshly pretreated MWCNTPE was modified by attaching ~0.2 ml of DNA stock solution (0.001 M) was dropped onto the surface of the electrode, also followed by drying at room temperature for 6 h. Finally, it was soaked in double distilled water for more than 3 h to remove unabsorbed ds-DNA.

3. Results and discussion

3.1. Electrochemical behaviour of DNA at MWCNTPE

When a solution of DNA (6.66×10^{-5} M) in 0.2 M acetate buffer of pH 6.3 ± 0.01 was electrolysed using MWCNTPE, it produced two oxidation peaks (Fig. 1) at + 0.75 and + 1.09 V respectively. The peak current was found to be proportional to DNA concentration in the analyte. The first peak at + 0.75 V may be due to the oxidation of guanine and second peak at + 1.09 V is due to the oxidation of adenine site of DNA molecule. Significantly ds-DNA did not produce any reduction peak under the given conditions.

3.2. Electrochemical characterization of MWCNTPE modified by ds-DNA

Fig. 2 presents the cyclic voltammogram (CV) of MWCNTPE and ds-DNA–MWCNTPE in 3.0×10^{-3} M $K_3[Fe(CN)_6]$ solution containing KCl supporting electrolyte. As DNA adsorbed on the MWCNTPE and DNA were both negatively charged, showed a repellence towards $[Fe(CN)_6]^{4-}$, leading to a decrease of peak currents on ds-DNA–MWCNTPE. These electrochemical behaviours indicated that the electrode surface had been covered with DNA. After a number of sweeps, CV of ds-DNA–MWCNTPE in $K_3[Fe(CN)_6]$ solution was almost unchanged, indicating its good structural stability.

3.3. Electrochemical behaviour of vitamin B_1 on ds-DNA–MWCNTPE

Fig. 3 shows the DPV curves of MWCNTPE and ds-DNA–MWCNTPE in 1.0×10^{-4} M VB_1 and 0.2 M acetate buffer solutions with pH value 6.3. As can be clearly seen, there is a reduction peak at −1.42 V on MWCNTPE, while the reduction peak current decreased significantly on ds-DNA–MWCNTPE.

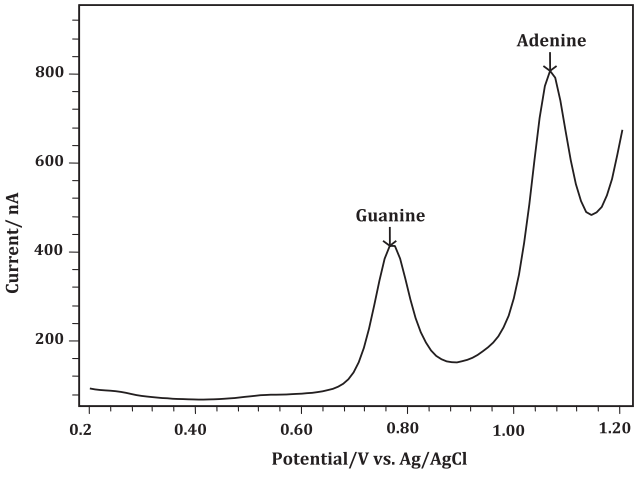


Figure 1 Differential pulse voltammograms of 6.66×10^{-5} M DNA in acetate buffer of pH 6.3 at MWCNTPE, scan rate 50 mVs^{-1}, current range 10 μA and pulse amplitude 50 mV.

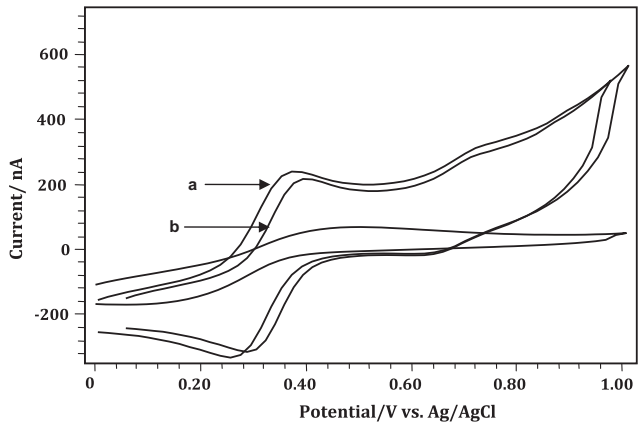


Figure 2 CV of MWCNTPE (curve a) and ds-DNA–MWCNTPE (curve b) in 3.0×10^{-3} M $K_3[Fe(CN)_6]$ solution containing 0.2 M acetate buffer; scan rate 50 mVs^{-1}.

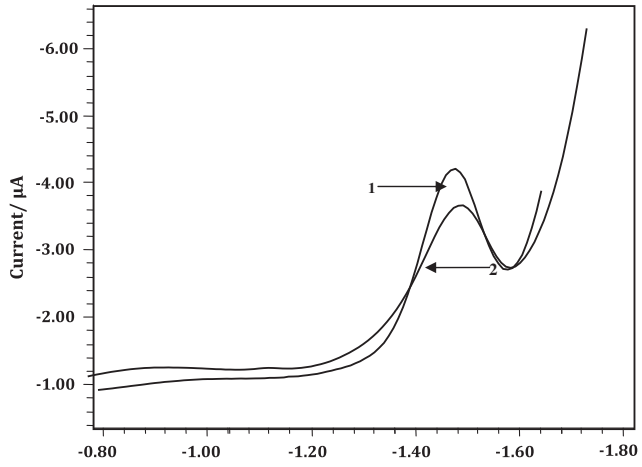


Figure 3 Curve (1) differential pulse voltammograms of 1.0×10^{-4} M vitamin B_1 at bare MWCNTPE in 0.2 M acetate buffer (pH 6.3 ± 0.01), curve (2) DPV of 1.0×10^{-4} M vitamin B_1 at DNA modified MWCNTPE, pulse amplitude 50 mV, scan rate 50 mVs^{-1}, current range 10 µA.

The evident difference in current intensity demonstrated the existence of the interaction between DNA and VB_1. It is considered that the only reason is the formation of the electrochemical inactive super molecular complex as demonstrated by using the electrochemical method.

3.4. Interaction of vitamin B_1 with DNA in solution

After studying the electrochemical behaviour of vitamin B_1 at the MWCNTPE, we have been able to study its interaction with DNA. For the said purpose experimental sets of solutions were prepared by taking a fixed concentration of 1.0×10^{-4} M vitamin B_1 in 0.2 M acetate buffer of pH 6.3, and varying the concentration of DNA from 6.66×10^{-5} M to 2.666×10^{-4} M. The reduction of vitamin B_1 was investigated for each set. Fig. 4 shows DPV with and without adding DNA into vitamin B_1 solution. The peak current decreases and peak potential was shifted towards a more negative value of the applied potential with the increase of DNA concentration. Probably, this

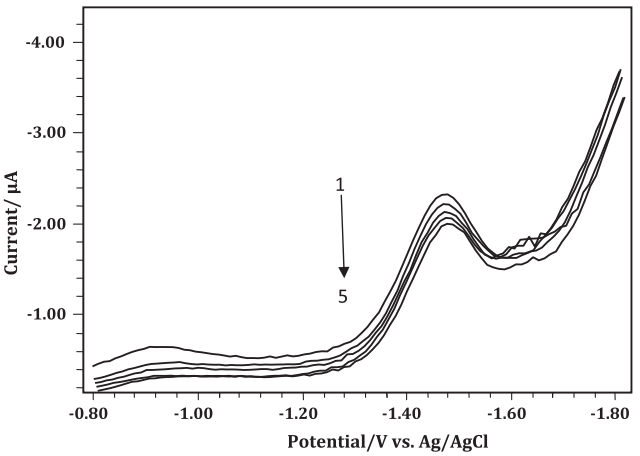


Figure 4 DPV of 1.0×10^{-4} M of vitamin B_1 in the absence (1) and the presence of (2) 6.6×10^{-5} M (3) 1.33×10^{-4} M (4) 2.0×10^{-4} M (5) 2.66×10^{-4} M DNA in 0.2 M acetate buffer of pH 6.3 at MWCNTPE, pulse amplitude 50 mV, scan rate 50 mVs^{-1}, current range 10 µA.

current decrease can be ascribed to the DNA–VB_1 complexation in the "bulk" solution. Bard et al. reported that positive peak potential shifts of intercalators were observed in the binding form via hydrophobic interactions (intercalation) while electrostatic interactions led to negative shifts.

After interaction with different concentrations of DNA, the peak current of vitamin B_1 attached to electrodes is plotted as a function of the concentration of DNA (Fig. 5).The magnitude of the peak current decreased sharply with the concentration of DNA. When the value of [DNA] reaches to 1.0×10^{-4} M the decrease in peak current becomes slight. At 1.6×10^{-4} M DNA, the current becomes saturated and remains constant indicating that all the accessible VB_1 attached to the electrode surface were bound to DNA completely. This illustrates that VB_1 attached to the electrode interacts with DNA quantitatively, and the interactive equilibrium is reached at the [DNA] value of 1.5×10^{-4} M.

3.5. The binding constant and the binding number between DNA and VB_1 on MWCNTPE

There are three models of binding of small molecules to DNA double helix viz., intercalative binding, groove binding and electrostatic binding. Bard et al. have reported the negative shift and positive shift in the peak potential for electrostatic and hydrophobic (intercalation) interactions between a ligand and DNA. A negative shift in the peak potential of vitamin B_1 was observed in the presence of DNA indicating the presence of electrostatic interactions.

The binding constant (β) and the binding number (n) between DNA and VB_1 were calculated according to the literature (Liping et al., 2006; Yaheng et al., 2008; Nawaz et al., 2006). Under the assumption that VB_1 and DNA formed the compounds DNA–$n$$VB_1$, the following equilibrium equation existed.

$$DNA + nVB_1 \rightarrow DNA(VB_1)n$$

The equilibrium constant is

$$\beta = [DNA - nVB_1]/[VB_1]n[DNA]$$

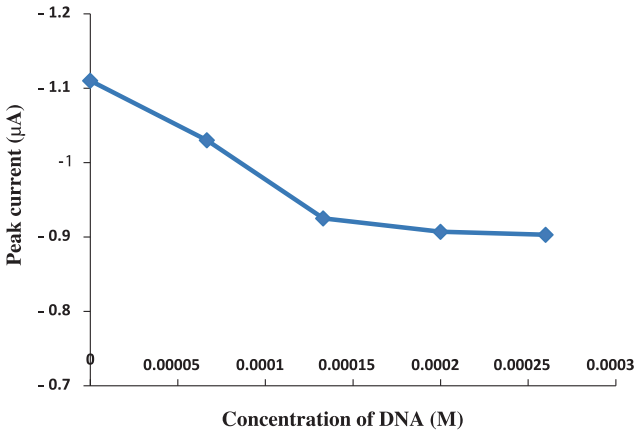


Figure 5 Relationship between peak current and concentration on addition of DNA in acetate buffer (pH = 6.3).

and the following equation can be deduced:

$$\log[\Delta I/\Delta I_{max} - \Delta I] = \log \beta + n \log[\text{DNA}]$$

where ΔI is the difference in peak current in the presence and absence of DNA and ΔI_{max} corresponds to the obtained value when the concentration of VB_1 is extremely higher than that of DNA. If DNA interacts with vitamin B_1 to form a single complex, then the plot of log $[\Delta I/\Delta I_{max} - \Delta I]$ versus log[DNA] (Fig. 6) shows linearity. The values of binding constant and binding ratio are obtained from intercept and slope, respectively and these values are found to be 5.097 M^{-1} and 2.

3.6. Spectroscopic evidence of the interaction between DNA and VB_1

UV–vis spectroscopic techniques have often been used to study the drug–DNA interaction mechanism.(Liping et al., 2006; Yaheng et al., 2008; Shankara et al., 2009) Fig. 7 shows the UV spectra of vitamin B_1 (curve 1), DNA (curve 2) and VB_1–DNA (curve 3). DNA has an absorption peak at about 260 nm. Vitamin B_1 has two absorption peaks at about 230 and 270 nm, respectively. Having interacted between DNA and vitamin B_1, one big absorption peak has been observed at about 265 nm, which ascribes to the combination of DNA and vitamin B_1. Therefore, it could be concluded that vitamin

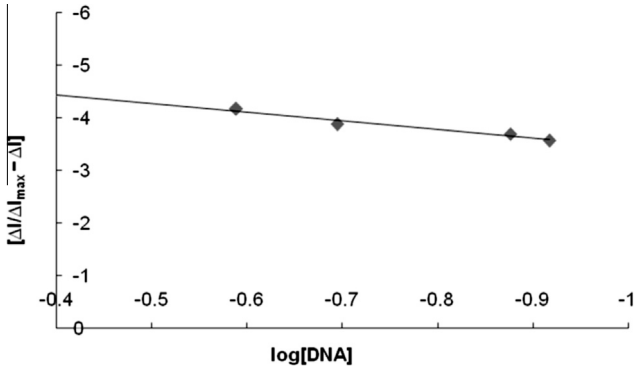


Figure 6 The relationship between $[\Delta I/\Delta I_{max} - \Delta I]$ and log[DNA] at MWCNTPE in 0.2 M acetate buffer solution (pH 6.3 ± 0.01) in the absence and the presence of DNA.

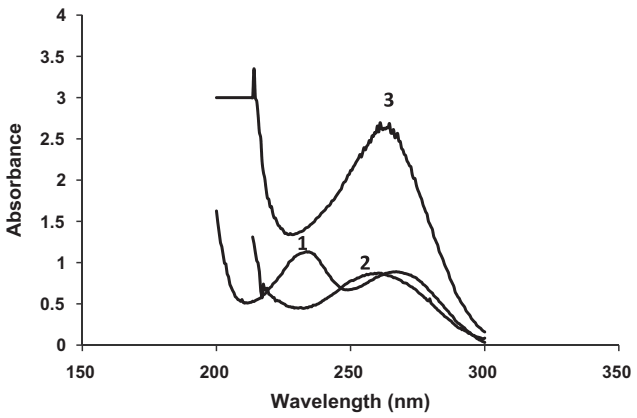


Figure 7 UV spectra of (1) 2.0×10^{-4} M vitamin B_1, $(22.0 \times 10^{-4}$ M ds-DNA and (3) mixture of ds-DNA–vitamin B_1 $(2.0 \times 10^{-4}$ M: 2.0×10^{-4} M).

B_1 interacts with DNA. The result is consistent with that from electrochemical study.

4. Conclusion

This paper investigated the interaction of vitamin B_1 with DNA by electrochemistry and UV spectral study. From the electrochemical and UV–vis spectral studies, it was inferred that an electrochemical inactive complex DNA–nVB_1 was formed. The binding constant of this complex was calculated to be 5.097 M^{-1}, and the binding number was determined to be 2 between DNA and vitamin B_1. The results indicate the electrostatic interaction between DNA and vitamin B_1.

References

Akyilmaz, E., Yaoa, A., Dinçkaya, E., 2006. Whole cell immobilized amperometric biosensor based on *Saccharomyces cerevisiae* for selective determination of vitamin B_1 (thiamine). Anal. Biochem. 354, 78–84.

Brahman, P.K., Dar, R.A., Tiwari, S., Pitre, K.S., 2012. Electrochemical behaviour of gatifloxacin at multi-walled carbon nanotube paste electrode and its interaction with DNA. Rev. Anal. Chem. 31, 83–92.

Dogan-Topal, B., Ozkan, Sibel A., 2011. A novel sensitive electrochemical DNA biosensor for assaying of anticancer drug leuprolide and its adsorptive stripping voltammetric determination. Talanta 83, 780–788.

Liping, W., Lin, L., Ye, B., 2006. Electrochemical studies of the interaction of the anticancer herbal drug emodin with DNA. J. Pharm. Biomed. Anal. 42, 625–629.

Lu, X., Zhou, X., Chen, J., Kang, J., Gao, J., 2000. Voltammetry of the erythromycin-modified glassy carbon electrode and interaction with DNA. Anal. Lett. 33, 193–207.

Markopoulou, C.K., Kagkadis, K.A., Koundourellis, J.E., 2002. An optimized method for the simultaneous determination of vitamins B1, B6, B12, in multivitamin tablets by high performance liquid chromatography. J. Pharm. Biomed. Anal. 30, 1403–1410.

Nawaz, H., Rauf, S., Akhtar, K., Khalid, A.M., 2006. Electrochemical DNA biosensor for the study of ciprofloxacin–DNA interaction. Anal. Biochem. 354, 28–34.

poongothai, S., Ilavarasan, R., Karrunakaran, C.M., 2010. Simultaneous and accurate determination of vitamins b_1, b_6, b_{12} and alphalipoic acid in multivitamin capsule by reverse–phase high performance liquid chromatographic method. Int. J. Pharm. Pharm. Sci. 2.

Radi, A., 2010. Application of electrochemical methods for analysis of fluoroquinolones antibacterial agents and fluoroquinolones–DNA interactions. Open Chem. Biomed. Methods J. 3, 27–36.

Raufa, S., Gooding, J.J., Akhtar, K., Ghauria, M.A., Rahman, M., Anwar, M.A., Khalid, A.M., 2005. Electrochemical approach of anticancer drugs–DNA interaction. J. Pharm. Biomed. Anal. 37, 205–217.

Shankara, S.K., Katrahalli, U., Seetharamappa, J., 2009. Electrochemical studies and spectroscopic investigations on the interaction of an anticancer drug with DNA and their analytical applications. J. Electroanal. Chem. 636, 93–100.

Wang, S., Peng, T., Yang, C.F., 2003. Investigation of the interaction of DNA and actinomycin D by cyclic voltammetry. J. Electroanal. Chem. 544, 87–92.

Yaheng, X.T., Dong, S.H., Ye, B., 2008. Interaction of anticancer herbal drug berberine with DNA immobilized on the glassy carbon electrode. Bioelectrochemistry 73, 18–22.

Zhang, N., Xu, C., Wei, Q., Du, B., Li, R., Zhang, T., Wu, D., Dai, Y., 2010. Studies on the interaction of DNA with vitamin B_{12} based on the immobilization of ds-DNA on nano-scale hydroxyapatite coating. Adv. Mat. Lett. 1, 34–39.