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Electrochemical and Ultraviolet–visible spectroscopic studies on the interaction of deoxyribonucleic acid with vitamin B_6

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ABSTRACT

Studies on the interaction of DNA with vitamin B_6 were carried out with a DNA-modified electrode by electrochemistry and Ultraviolet–visible spectroscopy. The results showed that there exists the supra-molecule interaction between base groups on DNA and vitamin B_6 by forming hydrogen binding, the binding equilibrium constant of the interaction is equal to 115.3 M^{-1}, the binding ratio of nucleotide to vitamin B_6 is 5:1. Based on the electrochemical and Ultraviolet–visible spectrum studies the interaction mode of DNA with vitamin B_6 was explored.

1. Introduction

Much attention was paid to the interaction of deoxyribonucleic acid (DNA) with small molecules, especially drug molecules owing to the importance for the rational design and construction of new and more efficient drugs targeted to DNA [1,2]. It is known that the interactions of DNA with small molecules have three models: (I) Electrostatic interaction, which is from the negative-charged nucleic sugar-phosphate structure; (II)binding hydrophobically against the minor groove; and (III) intercalation between the stacked base pairs of native DNA [3,4]. Vitamin B_6 is a water-soluble vitamin that exists in three major chemical forms: pyridoxol, pyridoxal, and pyridoxamine. It performs a wide variety of functions in one's body and is essential for one's good health. For example, vitamin B_6 is needed for more than 100 enzymes involved in protein metabolism. One's body needs vitamin B_6 to make hemoglobin, which carries oxygen to tissues. A vitamin B_6 deficiency can result in a form of anemia that is similar to iron deficiency anemia. Vitamins are important to one's immune defenses because they promote the growth of white blood cells that directly fight infections. Animal studies showed that a vitamin B_6 deficiency can decrease the antibody production and suppress the immune response [5]. Recent researches showed that DNA damage from deficiencies of the micronutrient including vitamin B_6, Vitamin B_{12}, etc., is likely to be a major cause of cancer [6],and vitamin B_6 has a crucial role in 1-carbon metabolism, which involves DNA synthesis and DNA methylation [7] and can modulate gene expression [8]. So studies on the interaction of DNA with vitamin B_6 are helpful to understand its physiological action. To the best of our knowledge, the electrochemical study on the interaction of DNA with vitamin B_6 has not been presented although studies on the interaction of DNA with some drugs [9–15] have been reported.

We formerly prepared ZrO_2 gel-derived DNA-modified electrode [16,17], by which the effect of lanthanide on DNA electron transfer behavior and the interaction of DNA with 2,2'-bipyridine were studied with the aid of electrochemical probe molecules $Fe(CN)^{3-}$ and $Co(phen)_3^{3+}$, respectively. Now we continue to study on the interaction of DNA with small molecule. Pyridoxol, which the structure is expressed as follows in Fig. 1, is chosen as a representative of vitamin B_6 for investigating the interaction of DNA with vitamin B_6 by a DNA-modified electrode.

2. Experimental

2.1. Chemicals

Salmon deoxyribonucleic acid (DNA) was obtained from Sigma(USA) and used as received. Stock solutions of DNA were prepared by dissolving an appropriate amount of DNA in distilled water and stored at 4°C. The concentration of the stock solution of DNA was determined by ultraviolet absorbance at 260nm using the molar extinction coefficient = 6600$M^{-1}cm^{-1}$ [18], the concentration of 2.0mg/mL dsDNA solution is equal to 5.1×10^{-3} M denoted by nucleotide of DNA. Generally, the stock solution of DNA is fresh for test, the storage time is not longer than 96h. Single strand DNA (ssDNA) was obtained by heating a double-

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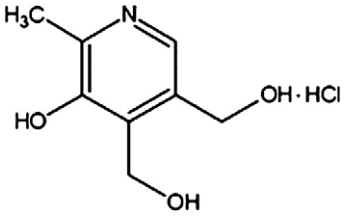

Fig. 1. The structure of pyridoxol.

strand DNA (dsDNA) solution to 100°C for 30min and suddenly immersing it into a cool water. 1,10-phenanthroline monohydrate and $CoCl_2{\cdot}6H_2O$, from Second Chemical Reagent Factory of Shanghai, were of analytical reagent grade. The probe molecule tris(1,10-phenanthroline) cobalt(III) perchlorate trihydrate [$Co(phen)_3(ClO_4)_3{\cdot}3H_2O$] was prepared according to previously reported procedures [19]. All other chemicals (Tris–hydroxymethyl-aminoethane, NaCl) were of analytical reagent grade without further purification. Vitamin B6, a medicine injection solution, was from Jinan Limin Pharmaceuticals CO.,LTD, China. The buffer solution refers to 0.10M tris–HCl buffer solution with pH 7.20 containing 5mM NaCl supporting electrolyte.

2.2. Apparatus

The cyclic voltammetric (CV) experiments were carried out with a CHI820B electrochemical workstation (CH Instrument Company, Texas, USA) using a three-electrode system at room temperature (25°C). A DNA-modified electrode was used as the working electrode, and a platinum sheet as the counter electrode, a saturated calomel electrode (SCE) as the reference electrode. All potentials are reported *vs* SCE. Ultraviolet–visible absorbance spectra were conducted with a double beam UV-8500 spectrophotometer, which was from Tianmei scientific equipment limited company of Shanghai, China.

2.3. Preparation of DNA-modified electrode

A glassy carbon (GC) disk electrode of 3.0mm diameter was used as a matrix of the working electrode. The GC electrode was polished using a piece of 1200 diamond paper and then 0.3 to 0.05μm alumina by turns. Finally, the GC electrode was washed with absolute alcohol and distilled water in an ultrasonic bath for 5min, respectively. Thus a mirror-like and shiny electrode surface was obtained.

DNA-modified electrodes were prepared like procedures described by Oliveira Brett and Yang [20–23]. 10μL dsDNA solution (5.1×10^{-3} M) was dropped on the mirror-like GC electrode surface in air at 4°C for 10h for drying, the resulting electrode was denoted as a dsDNA-GC electrode. Before use the electrodes were immersed in buffer solution for 15min, then rinsed lightly with distilled water for the removal of unadsorbed DNA, such the dsDNA-GC electrodes were used as working electrode for test.

DNA on GC electrode surface was removed by means of erasure with a sheet of velveteen and it was sonicated in distilled water with an ultrasonic cleaner for 15min for restoration of the GC electrode.

3. Results and discussion

3.1. Electrochemical characterization of DNA-modified electrode

Transition-metal complexes are often applied to probe both structural and functional aspects of nucleic acid chemistry [3], so the species are called probe molecule. In order to characterize the interaction of DNA with vitamin B_6 $Co(phen)_3^{3+}$, a cobalt complex, was used as electrochemical probe molecule [24].The above DNA working

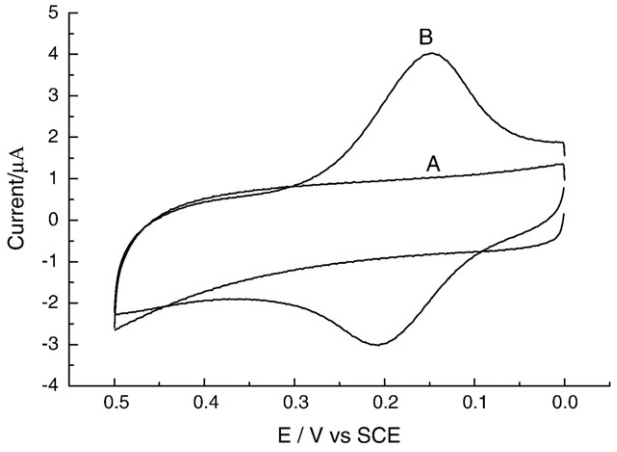

Fig. 2. Cyclic voltammograms of electrodes in 5.0 mM pH = 7.20 tris–HCl buffer solution containing 5.0 mM NaCl without $Co(phen)_3^{3+}$ probe molecule. A: the curve of a bare glassy carbon electrode in the buffer solution after immersed in 5.0×10^{-4} M $Co(phen)_3^{3+}$ probe molecule for 15 min. B: the curve of a dsDNA-GC electrode in the same buffer solution after immersed in 5.0×10^{-4} M $Co(phen)_3^{3+}$ probe molecule for 15 min. Potential range between 0.00 V and 0.50 V at a sweep rate of 20.0 mV/s.

electrode was immersed in 5.0×10^{-4} M $Co(phen)_3^{3+}$ solution for absorbing the probe molecule for 15min. Then the dsDNA electrode was taken out and rinsed with distilled water. Finally, the electrode was taken into buffer solution for electrochemical experiment. In order to identify the dsDNA immobilized on the glassy carbon electrode, a control electrochemical experiment was conducted with the bare glassy electrode, which was also immersed in 5.0×10^{-4} M $Co(phen)_3^{3+}$ solution for 15min before use. The resulting voltammograms were shown in Fig. 2.

From Fig. 2 the bare glassy electrode has no probe molecule current in the buffer solution while the DNA-modified electrode has a couple of clear probe molecule currents in the same buffer solution. This showed that the dsDNA was immobilized onto the glassy carbon electrode. Although free guanine, adenine, thymine and cytosine (DNA base groups) can be oxidized electrochemically in solution [25], no peak current of DNA-modified electrode was observed during electrochemical scanning in the 0.0V–0.5V potential rang in the buffer (see curve A in Fig. 2). The current of dsDNA electrode is attributed to the electron transfer of the probe molecule absorbed on

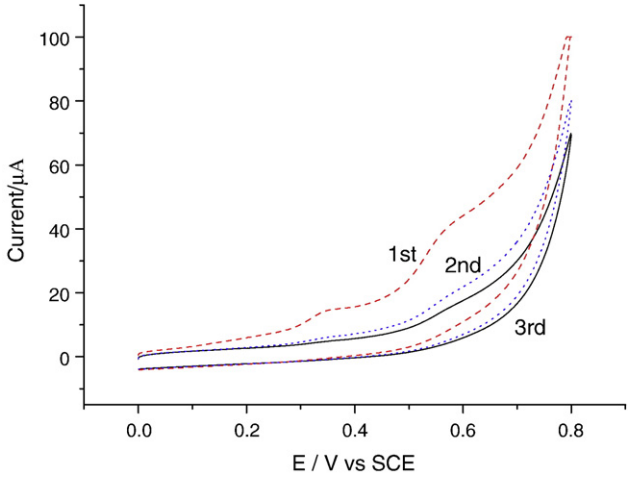

Fig. 3. A DNA-modified electrode in 0.10 M NaOH solution. Potential range between 0.00 V and 0.80 V at a sweep rate of 20.0 mV/s.

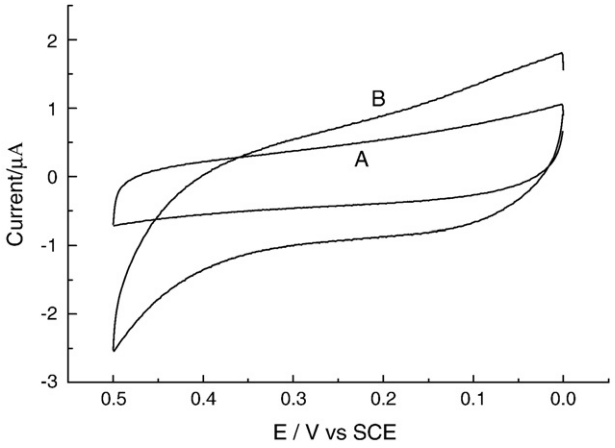

Fig. 4. Cyclic voltammograms of GC-dsDNA electrode. A: dsDNA-GC electrode in 5.0 mM pH=7.20 tris–HCl buffer solution containing 5.0 mM NaCl without vitamin B_6; B: dsDNA-GC electrode in the same buffer solution with 0.0187 M vitamin B_6. Potential range between 0.00 V and 0.50 V at a sweep rate of 20.0 mV/s.

dsDNA, but not to redox of DNA base groups. The main reason that dsDNA was not oxidized electrochemically in the neutral buffer solution is as follows. First, the glassy carbon electrode has poorly electrochemical response for the base groups of nucleic acids [26–28]. Second, the base pairs of adenine, thymine, cytosine, guanine are packed inside the double-stranded rigid helix of DNA, so that it is difficult to transfer electron inside to the electrode surface [21,29,30]. Only when the base pairs are exposed outside by heating DNA [31] or treating DNA with strong acid or alkali [32], or heavy metal ions [21], does the electro-oxidation of these base groups take place easily. It is a fact that the DNA-modified electrode has no oxidized peak current even if the positive potential was scanned to 1.20V in this neutral buffer in our diagnostic experiments. However, the DNA-modified electrode has an oxidized current at 0.58V in 0.1M NaOH solution as shown in Fig. 3. Because guanine, the easiest oxidizable of all DNA bases, is oxidized at 0.7V in pH 7.4 solution [33,34], and its oxidative potential is influenced with solution pH [35], the oxidative peak at 0.58V in Fig. 3 corresponds to the electrochemical oxidization of guanine. The alkaline solution made the DNA immobilized on GC surface denatured [32], which caused the base group exposed to outside and oxidized electrochemically at 0.58V. When the electrochemical scan was repeated a second time, the guanine on the GC electrode was electro-oxidized again with a decline of peak current, until the peak current disappeared the third time. That means the guanine on GC electrode was oxidized thoroughly after the second scan was performed.

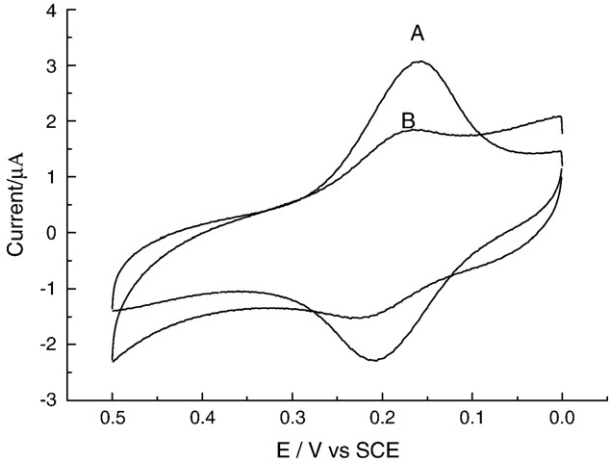

Fig. 5. Cyclic voltammograms of a dsDNA-GC electrode in 5.0×10^{-4} M $Co(phen)_3^{3+}$ pH=7.20 solution containing 5.0 mM NaCl supporting electrolyte without vitamin B_6 (A) and with 0.0187 M vitamin B_6 (B). Potential range between 0.00 V and 0.50 V at a sweep rate of 20.0 mV/s.

3.2. Interaction of dsDNA with vitamin B_6

In order to investigate the interaction of DNA with vitamin B_6 molecule, a dsDNA-GC electrode was taken in buffer solution in the present of vitamin B_6 and in the absence of vitamin B_6 for a voltammetric test. The resulting volammograms are shown in Fig. 4.

No peaks can be seen during electrochemical scanning in the present and absence of vitamin B_6 as shown in Fig. 4 although the background currents are larger in the presence of vitamin B_6 than in its absence. The increases of charged and uncharged currents in the vitamin B_6 solution suggested that vitamin B_6 molecule probably interacts with DNA. In order to confirm the interaction the DNA-modified electrode was scanned in buffer solution containing 5.0×10^{-4} M $Co(phen)_3^{3+}$ probe molecule, then vitamin B_6 was added into the test solution. The experiments showed the peak current of probe molecule decreased as vitamin B_6 was added into the test solution. The more vitamin B_6 was added, the more the peak current of probe molecule decreased. Thus, when the concentration of vitamin B_6 was adjusted to 0.0187M, the peak current decreased by 47.2% with respect to original peak current, as showed in Fig. 5.

The phenomenon confirmed that vitamin B_6 molecule interacts with dsDNA. Since the pKa_1 of pyridoxol is equal to 5.0, pKa_2 9.0, pyridoxol could be protonated in pH < 5.0 acidic solution [36,37] and charged negatively in pH > 9.0 alkaline solution. By assuming that vitamin B_6 molecule was not protonated in the mostly neutral buffer solution of pH 7.20, the electrostatic interaction between DNA and vitamin B_6 could be ignored. Since the probe molecule $Co(phen)_3^{3+}$ gets in double-strand of DNA [3,24], the vitamin B_6 molecule competes for sites at DNA with probe molecule, which leads to a decrease in the peak current of the probe molecule when vitamin B_6 is added to the test solution. Thus, it can be deduced that vitamin B_6 also gets in the double-stranded DNA.

Many electrochemical studies on vitamin B_6 have been performed by using both gold [36,38] and glassy carbon electrodes [39,40] since the determination of pyridoxine was described for the first time in 1975 [41]. In our experiments the voltammetric peaks of DNA and

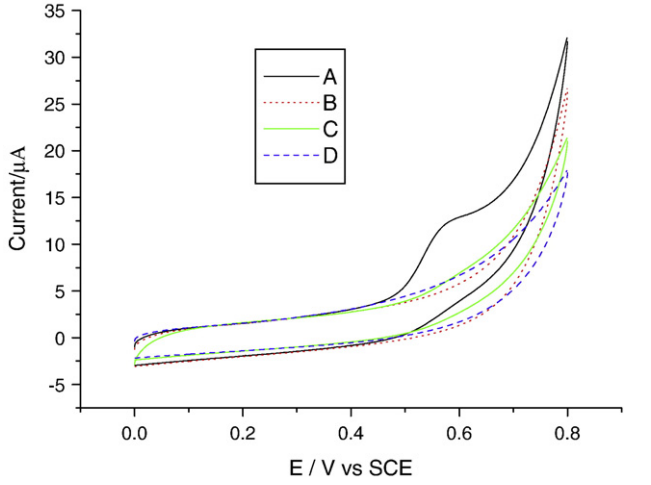

Fig. 6. Cyclic voltammetric curves of a DNA-modified GC electrode in 0.10 M NaOH+ 0.2433 mM vitamin B_6 solution (A), the DNA-modified GC electrode in 0.10 M NaOH supporting electrolyte without vitamin B_6 (B). Cyclic voltammetric curves of the bare GC electrode in 0.10 M NaOH+0.2433 mM vitamin B_6 solution (C), the bare GC electrode in 0.10 M NaOH without vitamin B_6 solution (D). Potential range between 0.00 V and 0.80 V at a rate of 20.0 mV/s.

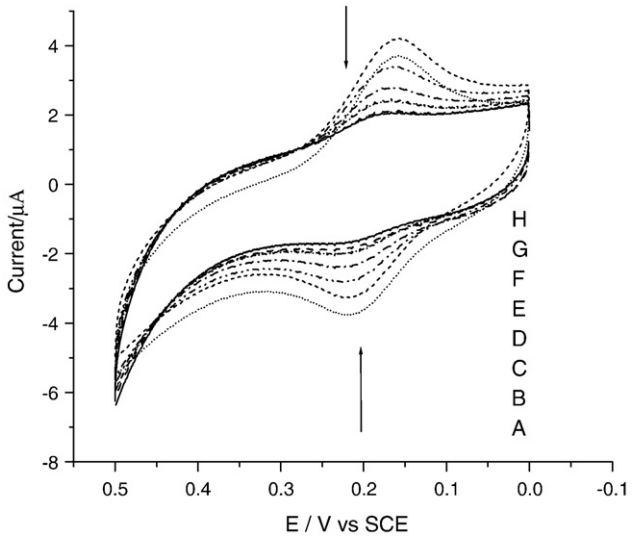

Fig. 7. Cyclic voltammetric curves of dsDNA-GC electrode in 5.0×10^{-4} M $Co(phen)_3^{3+}$ pH = 7.20 buffer solution with 5.0 mM NaCl supporting electrolyte in the potential range between 0.00 V and 0.50 V at a sweep rate of 20.0 mV/s with different concentrations of vitamin B_6 A: 0.0116 M, B: 0.0221 M, C: 0.0268 M, D: 0.0317 M, E: 0.0355 M, F: 0.0405 M, G: 0.0487 M, H: 0.060 M.

vitamin B_6 were not observed in pH7.20 buffer solution even if the sweep potential window was set up between 0.00 and 1.20V. However, in 0.10M NaOH alkaline solution the electro-catalysis of DNA-modified electrode on vitamin B_6 was observed during the electrochemical scanning as shown as curve A in Fig. 6, the same GC electrode without DNA has no voltammetric peaks in this NaOH solution containing vitamin B6. In alkaline solution both DNA and vitamin B_6 are charged negatively [42,36–38], they should have repelled and been far away from each other, but the electro-catalysis of DNA on vitamin B_6 can take place in the alkaline solution. The phenomena confirmed further that DNA interacts with pyridoxol. The repetitious experiments showed after the DNA was removed from the GC electrode surface the electrochemical signal of vitamin B_6 disappeared, and the voltammetric peak at 0.58V appeared again when DNA was immobilized on GC electrode surface again. These phenomena showed the method for preparation of DNA-modified electrode described by Oliveira Brett et al. [20–23] is reliable and resulting DNA-modified electrode is stable and reproducible.

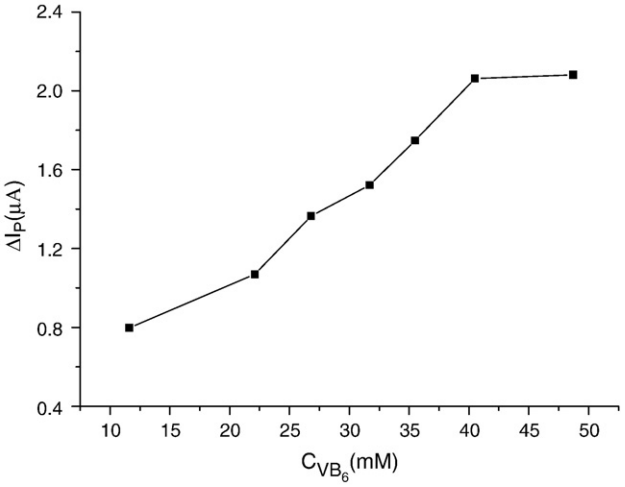

Fig. 8. The dependence of decrease value of the peak current on the concentration of vitamin B_6.

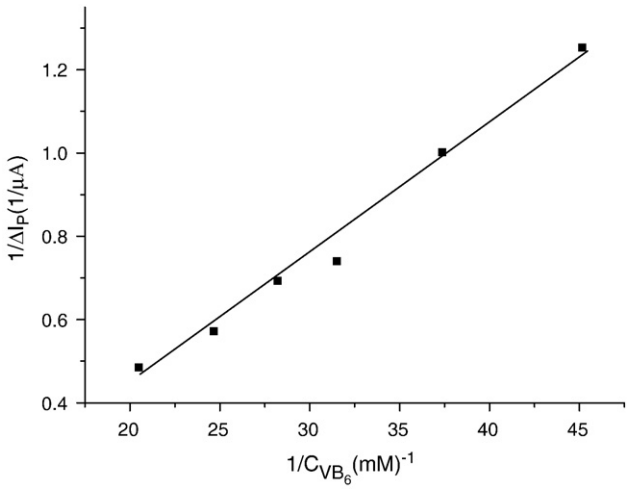

Fig. 9. The relationship between $1/\Delta I_P$ and $1/C$.

3.3. Binding equilibrium constant

Cyclic voltammetric experiments showed that DNA-modified electrode has a couple of stable voltammetric peaks from probe molecules when it was scanned in the probe solution. Further studies showed when vitamin B_6 was added into this solution, the peak current had an obvious decrease. And the higher the amount of vitamin B_6 added, the lower the peak current was. The curves are shown in Fig. 7.

When the concentration of vitamin B_6 reached 0.060M, the peak current no longer decreased. Fig. 8 shows the relationship between the decrease of the peak current and the concentration of vitamin B_6 added.

From Fig. 8 we can obtain an $1/\Delta I_p \backsim 1/C$ curve (Fig. 9). It is apparent that a good linear relationship($R = 0.9934$) exists between the reciprocal of the current drop and that of the vitamin B_6 concentration. This is in good agreement with Langmuir equation [17] below:

$$1/\Delta I_P = 1/\Delta I_{Pmax} + 1(\Delta I_{Pmax} kC)$$

where k is equilibrium constant of the interaction between vitamin B_6 and DNA, C is the concentration of vitamin B6, ΔI_p is the current drop and ΔI_p max stands for the maximum of the current drop. According to the above equation and the slope of the curve in Fig. 9, the binding equilibrium constant can be estimated at 115.3 M^{-1}.

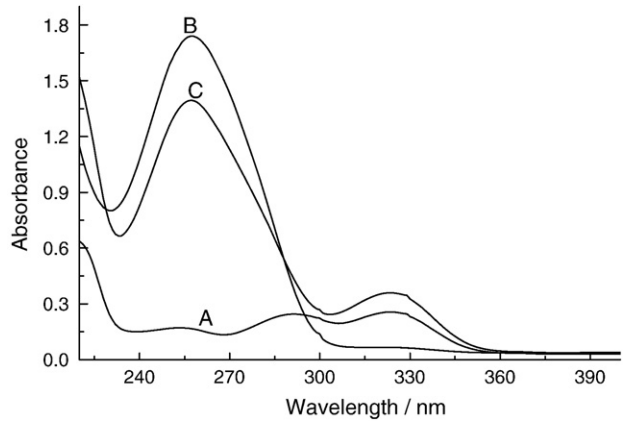

Fig. 10. Ultraviolet–visible Spectra of vitamin B6, DNA and their mixture. A: 5.0×10^{-5} M vitamin B_6; B: 2.5×10^{-4} M nucleotide(DNA); C: 2.5×10^{-4} M nucleotide+5.0×10^{-5} M vitamin B_6.

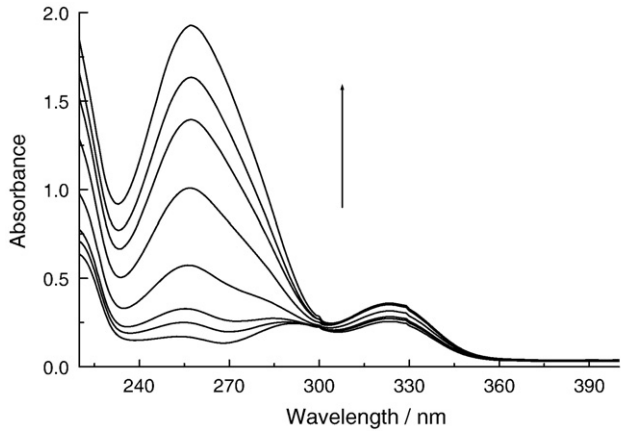

Fig. 11. Ultraviolet–visible absorbance spectra of dsDNA-Vitamin B_6 mixed solution. The concentration of vitamin B_6 always was 5.0×10^{-5} M, while the concentrations of nucleotide were 8.8×10^{-6} M, 1.8×10^{-5} M, 3.5×10^{-5} M, 8.8×10^{-5} M, 1.8×10^{-4} M, 2.5×10^{-4} M, 3.5×10^{-4} M, 4.4×10^{-4} M, respectively.

3.4. Spectroscopic studies and binding ratio

Ultraviolet–visible absorbance spectra of vitamin B_6, dsDNA and their mixture were measured, respectively, as shown in Fig. 10.

Vitamin B_6 has three absorbance peaks at 256nm, 291nm, 323nm (curve A). DNA has an absorption peak at 260nm, which is from nucleotide on the DNA strands. However, after DNA and vitamin B_6 were mixed, a clear decrease of absorbance at 260nm was observed (curve c).Meanwhile, an increase appeared at 323nm. The decrease at 260nm and increase at 323nm of the absorbance at spectra confirm further that vitamin B_6 interacts with DNA, indeed. Further studies showed that the absorbance value of the mixture increased gradually at first, up to a maximum value in 6min, then decreased slowly as time went by. For example, the absorbance of the mixture of 2.5×10^{-4} M DNA + 5.0×10^{-5} M vitamin B is equal to 1.60, 1.65, 1.63 at 3min, 6min, 15min, respectively. The increase of the absorbance is due to the uncoiling of double strands of DNA when vitamin B_6 was added at first, then vitamin B_6 began to bind to nucleotide on DNA strands, the absorbance began to descend again. In order to know specifically the ratio of nucleotide to vitamin B_6, the absorbance measurements of the mixture of different ratio were conducted with an Ultraviolet–visible spectrophotometer. The resulting curves are shown in Fig. 11.

In the experiments the concentration of vitamin B_6 was always kept at 5.0×10^{-5} M while the concentrations of DNA were adjusted according to the ratio that were needed. Then these solutions of mixtures were kept at 4°C for 24h for full completion of binding between nucleotide (from DNA) and vitamin B_6. The results showed that absorbance value at 323nm increased as the concentration of DNA increased. In the case of that the nucleotide concentration of DNA reached to 2.5×10^{-4} M (the molar ratio of nucleotide to vitamin B_6 is equal to 5:1), the absorbance value of the mixed solution at 323nm increased no longer while the absorbance at 260nm continued to increase with the addition of DNA, thus showing that the stoichiometric ratio between DNA and vitamin B_6 had been reached. This means that the binding ratio of nucleotide to vitamin B_6 is 5:1.

3.5. Interaction mode analysis

The interaction between DNA and vitamin B_6 is actually a supramolecular action by forming hydrogen bonds between base groups on DNA strands and hydroxyl groups and nitrogen atom on vitamin B_6 frame. The electrochemically diagnostic experiments showed that a dsDNA-GC electrode surface absorbed with probe molecule $Co(phen)_3^{3+}$ has a stable peak current in pH = 7.20 buffer solution with 5.0mM NaCl supporting electrolyte (without $Co(phen)_3^{3+}$ in this solution), which is due to the intercalation of aromatic heterocyclic complex probe molecule into DNA [3,4]. Similarly, this peak current of the same electrode disappeared gradually in the above same supporting solution as vitamin B_6 was added, which revealed that the vitamin B_6 molecules substituted for the probe molecules between double strands of DNA. Since the probe molecules intercalated into double strands of DNA, the substitute of vitamin B_6 for probe molecules intercalated, too. The other evidence for intercalation is that the absorbance of the solution increased when vitamin B_6 was added into dsDNA at first, then the absorbance decreased with time. The increase of the absorbance is attributed to the uncoil of the double strands of DNA, which leads to the exposure of the base groups on DNA to the incidence light for absorbance. After the double strands were uncoiled, vitamin B_6 molecules intercalated into the double strands by forming hydrogen bindings. At that time the strands started to close, the absorbance started to decrease again. The intercalation of vitamin B_6 into double-stranded DNA is responsible for the change of absorbance.

3.6. Conclusion

From the electrochemical and spectroscopic studies it is concluded that vitamin B_6 can interact with DNA, the binding equilibrium constant is equal to $115.3M^{-1}$, the binding ratio of nucleotide to vitamin B_6 is 5:1. The interaction will be helpful to understand the biological functions of vitamin B_6. The electro-catalysis of DNA on vitamin B_6 in the alkaline solution lays the foundation for development of analytical method for voltammetric determination of vitamin B_6.

Acknowledgements

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