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Interaction between tryptophan-vanillin Schiff base and herring sperm DNA

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Abstract: The interaction of the Schiff base (K[HL]) with herring sperm DNA was studied by UV–Vis absorption, fluorescence and viscosity methods in a physiological pH environment (pH 7.40), where the Schiff base was derived from vanillin and L-tryptophan. A binding ratio of *n*K[HL]:*n*DNA = 5:1 and an apparent molar absorption coefficient of ε(K[HL]–DNA) = 4.98×10^5 L mol^{-1} cm^{-1} were confirmed by the mole ratio method. The binding constants of $K_B^{\ominus}$(301 K) =1.94×10^5 L mol^{-1} and $K_B^{\ominus}$(310 K) = 1.09×10^5 L·mol^{-1} were obtained by the double reciprocal method. Thermodynamic parameters suggest that the interaction between K[HL] and DNA is driven mainly by enthalpy. Combined with Scatchard methods and viscosity methods, the results indicate the presence of intercalation and groove binding between K[HL] and DNA.

Keywords: vanillin; L-tryptophan; Schiff base; herring sperm DNA; interaction.

INTRODUCTION

The numerous hitherto performed biological experiments suggest that DNA is the primary intracellular target of an anticancer complex because the interaction between this molecule and DNA can cause DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death.[1,2] Generally, there are three modes for reversible binding of molecules with double-helix DNA in a non-covalent way: *i*) electrostatic interaction – electrostatic attractions with the anionic sugar-phosphate backbone of DNA, *ii*) groove binding–interactions with the DNA groove and *iii*) intercalation between the base pairs. Depending on the structural features of both the molecules and DNA, many molecules show more than a single interaction mode with DNA.[3]

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A Schiff base is obtained by the condensation reaction between an aldehyde and an amine. They have recently received considerable attention due to their good performance in coordination chemistry, unique anti-bacterial, anti-cancer, and other physical activities.[4–6] In particular, Schiff base complexes obtained from amino acids are finding applications in the understanding of many biochemical reactions *in vivo*. An amino acid is a kind of important biological ligand, which contains several N and O atoms. Cancerous cells have a much greater demand for amino acids than normal cells. Hence, amino acid Schiff base may deliver an anti-cancer base to cancerous cells, thereby increasing the selectivity of anti-cancer cells.[7]

L-Tryptophan (Trp) is an essential amino acid which is required for the biosynthesis of proteins. It is important in nitrogen balance and the maintenance of muscle mass and body weight in humans.[8]

In this study, a Schiff base containing an azomethine group was prepared by the condensation of vanillin with L-tryptophan, and then the interaction of the Schiff base with DNA was investigated systematically by spectroscopy and viscosity approaches. A series of thermodynamic parameters and binding constants were also obtained. The results are helpful to understand the interaction modes between the amino acid Schiff base and DNA.

EXPERIMENTAL

Apparatus

Carbon, hydrogen and nitrogen were obtained using a Vario El Cube instrument. The IR spectra (400–4000 cm^{-1}) were recorded as KBr pellets on a Spectrum One FTIR spectrophotometer. The absorption spectra were measured on an UV-210 spectrophotometer. The fluorescence spectra were recorded with a FL-4500 spectrofluorometer. The pH was measured using a pHS-2C digital pH-meter with a combined glass-calomel electrode.

Materials

Herring sperm DNA (hsDNA) was purchased from Sigma Biological Co. and used as received. The purity of the DNA was checked by monitoring the ratio of the absorbance at 260 and 280 nm. The ratio was 1.89, indicating that the DNA was free from protein.[9] The DNA was dissolved in doubly distilled deionized water with 50 mM NaCl and dialyzed for 48 h against a buffer solution at 4 °C. The concentration of the hsDNA stock solution was determined according to the absorbance at 260 nm using an extinction coefficients of 6600 L mol^{-1} cm^{-1}.

A tris-HCl buffer (pH 7.40) was used to control the pH of the reaction system. All of the samples were dissolved in the Tris-HCl buffer. L-Tryptophan was purchased from the Chengdu-China Kelong Chemical Plant (A.R.). Vanillin was purchased from the Xian-China Chemical Plant (A.R.). Acridine orange (AO) was purchased from the Shanghai-China Medicine Chemical Plant (A.R.). Other reagents were of at least analytical grade and were used without further purification.

Preparation of K[HL]

The Schiff base (K[HL]) derived from vanillin and L-tryptophan was prepared using a method similar to one given in the literature.[10] To a solution of vanillin (0.18 g, 1.2 mmol) in MeOH (10 cm^3), L-tryptophan (0.20 g, 1.0 mmol) in MeOH (15 cm^3) containing KOH (0.056 g, 1.0 mmol) was added. The obtained solution was then magnetically stirred for 5 h at 50–60 °C on a water bath. The volume of the obtained brownish red solution was reduced *in vacuo* using a rotary evaporator and then washed with absolute ethanol. After standing for several days, black crystals formed. Anhydrous diethyl ether was added to wash the black crystals and they were then dried in *vacuo* for 2 h.

Absorption spectral measurements

A solution (3 mL) containing an appropriate concentration of K[HL] in 1.0 cm quartz cells was titrated by successive additions of a certain concentration of DNA stock solution. The titration was performed manually using a micro-injector. Each addition was 10 μL to avoid a change in the volume. Appropriate blanks corresponding to the buffer were used as the reference. The absorption spectra were measured 5 min after each addition.

Fluorescence spectral measurements

A solution (3 mL) containing an appropriate concentration of K[HL]–DNA in 1.0 cm quartz cells was titrated using a stock solution of AO and a solution (3.0 mL) containing an appropriate concentration of AO–DNA in 1.0 cm quartz cells was titrated using a stock solution of K[HL]. The titrations were performed manually using a micro-injector. Each addition was 10 μL to avoid a change in the volume. The widths of both the excitation slit and the emission slit were set at 5.0 nm; and the excitation wavelength was set at 411.7 nm. The fluorescence emission spectra were measured 5 min after each addition.

Viscosity measurements

The viscosity experiments were realized using a viscometer which was immersed in a water-bath thermostated at room temperature. Different amounts of K[HL] were then added into the viscometer to give different values of c(K[HL]) while keeping the DNA concentration constant. The flow times of the samples were repeatedly measured with an accuracy of ±0.2 s using a digital stopwatch. Each point value was the average of at least three time measurements. The data are presented as $(\eta/\eta_0)^{1/3}$ *versus* c(K[HL]), where η and η_0 are the viscosity of DNA in the presence and absence of the Schiff base, respectively.

RESULTS AND DISCUSSION

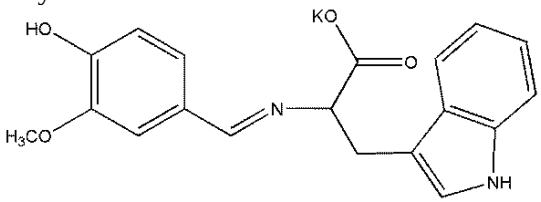
The IR spectrum of the black crystals of the synthesized Schiff base displayed clearly a strong band at 1627 cm^{-1} that can be assigned to the ν(C=N) azomethine stretching vibration. The symmetric carboxyl stretching $\nu_{sym}(COO^-)$ and the asymmetric carboxyl stretching $\nu_{asym}(COO^-)$ were at 1390 and 1593 cm^{-1}, respectively. The IR spectrum also showed a broad band at 3398 cm^{-1}, which can be attributed to the stretching vibration of the –OH group.[11] This indicates that a water molecule was present in the Schiff base.

Anal. Calcd. for $C_{19}H_{17}N_2O_4K{\cdot}2H_2O$: C, 55.34; H, 4.13; N, 6.80 %. Found: C, 55.91; H, 4.58; N, 6.61 %.

From the above results, the structure of K[HL] can be deduced (Scheme 1).

Absorption spectral studies

The binding of small molecules to DNA is classically characterized through absorption titrations.[12] Generally, a red shift (or blue shift) and hypochromic (or hyperchromic) effect are observed in the absorption spectra of small molecules if they intercalate with DNA.



Scheme 1. Structure of the Schiff base (K[HL]).

UV–Vis absorption spectra were obtained by titration of a K[HL] solution with increasing concentrations of DNA (Fig. 1). In the absence of DNA, the UV––Vis spectrum of K[HL] is characterized by three transitions: two higher energy absorptions bands at 213 and 280 nm; and a lower energy absorption band at 340 nm. With increasing concentration of DNA, the intensity of both the 213 and 280 nm absorbance peaks gradually increased, but the intensity of the 340 nm peak decreased. Isochromatic points were obtained at 317 and 375 nm.

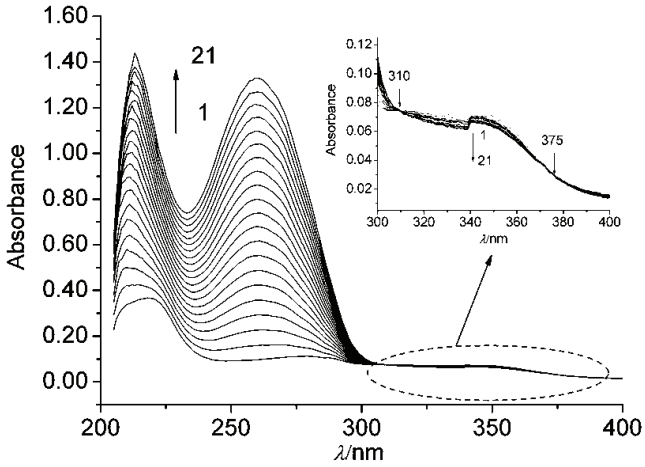


Fig. 1. Absorption spectra of K[HL] in different concentration of DNA (pH 7.40); for curve 1–21, c(K[HL]) = 1.00×10^{-5} mol L^{-1}, c(DNA) = 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26, 0.28, 0.30, 0.32, 0.34, 0.36, 0.38 and 0.40×10^{-5} mol L^{-1}, respectively.

A hypochromic effect and isosbestic points are evidence of DNA-intercalation.[13,14] The absorption spectra of K[HL] in the presence of DNA showed hypochromicity and isochromatic points, indicating the presence of intercalation between K[HL] and DNA.

In order to determine the stoichiometry for the formation of the K[HL]–DNA complex, the mole ratio method[15] was applied to the peak 213 nm. The mole ratio plots of DNA with K[HL] are shown in Fig. 2. The binding ratio of the complex was obtained: $n_{K[HL]}$:n_{DNA} = 5:1. According to the Lambert–Beer law: $A = \varepsilon bc$, where A is the absorbance of K[HL]–DNA, ε is the apparent molar absorption coefficient of K[HL]–DNA and c is the concentration of K[HL]–DNA. The apparent molar absorption coefficient of K[HL]–DNA was $\varepsilon = 4.98\times10^5$ L mol^{-1} cm^{-1}.

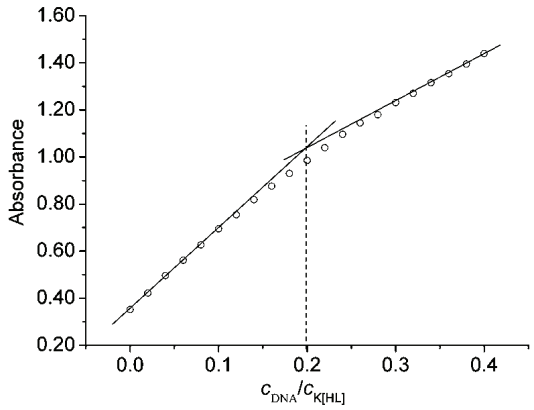


Fig. 2. Mole ratio plots of K[HL]–DNA in tris-HCl buffer (pH 7.40); c(K[HL]) = = 1.00×10^{-5} mol L^{-1}; c(DNA) = = 6.00×10^{-5} mol L^{-1}, λ = 213 nm.

Double reciprocal method

In order to determine the interaction between K[HL] and DNA, the binding constant was determined. The absorption relationship between the complex and DNA was expressed by the double reciprocal equation:[16–18]

$$1/(A_0 - A) = 1/A_0 + 1/(KA_0c_{DNA}) \quad (1)$$

where A_0 and A are the absorbances of K[HL] in the absence and in the presence of DNA, respectively. K is the binding constant between K[HL] and DNA and c_{DNA} is the concentration of DNA.

The double reciprocal plots of $1/(A_0 - A)$ *versus* $1/c_{DNA}$ were linear at 28 °C and 37 °C and the binding constants were calculated from the ratio of the intercept on the vertical (Fig. 3): $K_B^{\ominus}$(301 K) = 1.94×10^5 L mol^{-1}, $K_B^{\ominus}$(310 K) = = 1.09×10^5 L mol^{-1}. The above observed values are smaller than those of classical intercalators (ethidium–DNA,[19] 7×10^7 L mol^{-1}; proflavin–DNA,[20] 4.1×10^5 L mol^{-1}).

To obtain a detailed view of the interaction, the approach of parsing the free energy into component terms is a powerful and insightful method. The enthalpy change (ΔH) is considered as 0 for a small change of temperature.[21] The standard molar reaction enthalpy ($\Delta_r H_m^{\ominus}$), $K^{\ominus}$ and T are estimated from the following relationship:

$$\ln K_2^{\ominus}/K_1^{\ominus} = -\Delta_r H_m^{\ominus} (1/T_2 - 1/T_1)/R \quad (2)$$

where $K_1^{\ominus}$ and $K_2^{\ominus}$ are the standard binding constant of K[HL] and DNA at 28 and 37 °C, respectively, T_1 is 301.15 K, T_2 is 310.15 K. $\Delta_r H_m^{\ominus}$ is the standard molar reaction enthalpy. Then $\Delta_r H_m^{\ominus}$ is –49.7 kJ mol^{-1}. This result shows that the binding of K[HL] to DNA is exothermic.

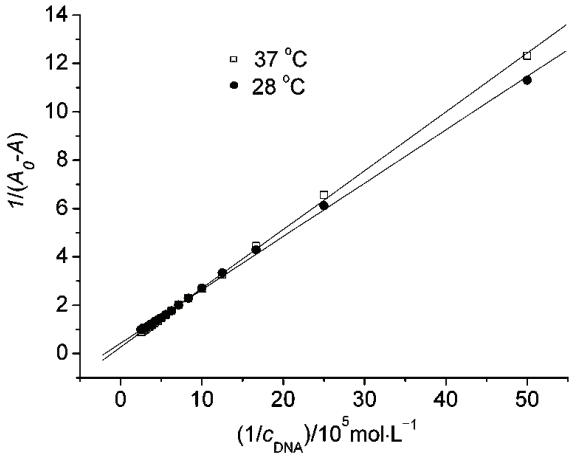


Fig. 3. Double reciprocal plots of K[HL]–DNA in tris-HCl buffer (pH 7.40) at 28 and 37 °C; c(K[HL]) = 1.00×10^{-5} mol L^{-1}.

The standard molar reaction Gibbs free energy ($\Delta_r G_m^{\ominus}$) and the standard molar reaction entropy ($\Delta_r S_m^{\ominus}$) are estimated from the following relationships:

$$\Delta_r G_m^{\ominus} = \Delta_r H_m^{\ominus} - T\Delta_r S_m^{\ominus} \quad (3)$$

$$\Delta_r G_m^{\ominus} = -RT \ln K^{\ominus} \quad (4)$$

where T is 301.15 K; $K^{\ominus}$ is the standard binding constant of K[HL] and DNA at 28 °C. $\Delta_r G_m^{\ominus}$ is –30.5 kJ mol^{-1} and $\Delta_r S_m^{\ominus}$ is –63.8 J mol^{-1} K^{-1}. The negative Gibbs free energy value indicates that the reaction between K[HL] and DNA is possible. The negative entropy value indicates that the degree of freedom of K[HL] is decreased after the binding, and that the DNA conformational freedom is also reduced upon K[HL]–DNA binding. It is obvious that the process of interaction of K[HL] and DNA is energetically highly favorable at room temperature and the binding reaction is driven mainly by enthalpy.[22]

Fluorescence measurements using Acridine Orange as a probe

Fluorescence is very useful method to investigate the interaction between small molecules and DNA. The fluorescence of DNA is weak, hence the utilization of fluorescence probes enables the study of interaction between small molecules and DNA. Acridine orange (AO) is a kind of cationic dye. Due to its planar aromatic chromophore, it can insert between two adjacent base pairs in a DNA helix.

The emission spectra of K[HL] bound to DNA in the absence and the presence of AO are given in Fig. 4. There was a significant increase in the fluorescence intensity and a red shift in the emission wavelength from 527 to 533 nm as a result of AO insertion between the base pairs of DNA.

If K[HL] has the same binding mode with DNA as AO, there will be competition between AO and the complex with DNA. Hence, the fluorescence spectrum will be changed.[23] The emission spectra of AO bound to DNA in the absence and the presence of K[HL] are given in Fig. 5. It could be seen that the fluorescence intensity of DNA–AO was efficiently quenched. This phenomenon suggested that K[HL] substituted for AO in the DNA–AO system, which led to a large decrease in the emission intensity of the DNA–AO system.

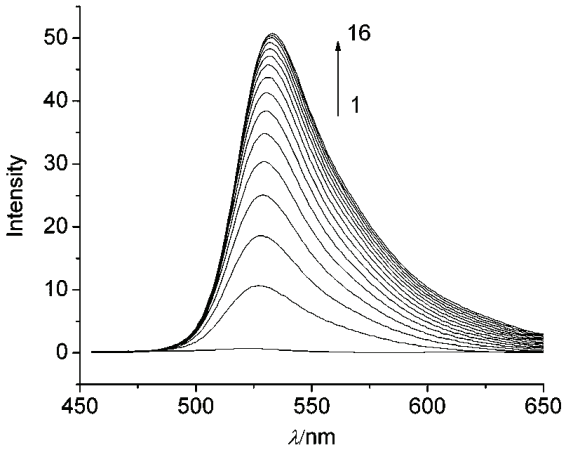


Fig. 4. Emission spectra of K[HL]–DNA in different concentrations of AO (pH 7.40; λ_{ex} = 411.7 nm); from curve 1–16, $c_{DNA} = 2.00\times10^{-5}$ mol L^{-1}, c_{AO} = 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26, 0.28, and 0.30×10^{-4} mol L^{-1}, respectively.

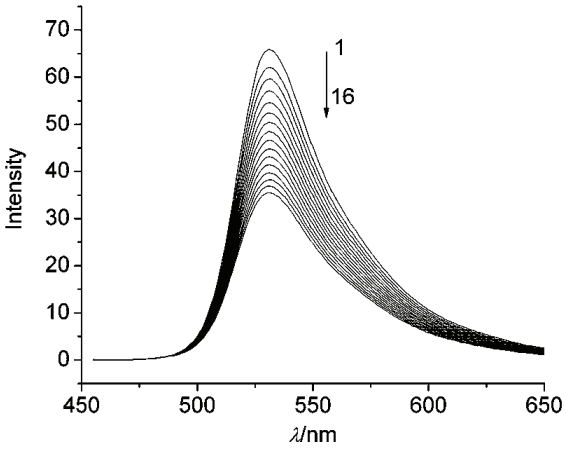


Fig. 5. Emission spectra of DNA–AO in different concentrations of K[HL] (pH 7.40; λ_{ex} = 411.7 nm); from curve 1–16, $c_{DNA\text{-}AO}$ = = 2.00×10^{-5} mol·L^{-1}, $c_{K[HL]}$ = 0.00, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14 and 0.15×10^{-3} mol L^{-1}, respectively.

From the changes in the emission spectra of Figs. 4 and 5, it can be seen that the competition between AO and K[HL] for DNA was remarkable. As the inter-

action between AO and DNA is intercalation, then the presence of intercalation between K[HL] and DNA is basically confirmed.[24]

Scatchard method

The binding mode between small molecules with DNA can be determined using the Scatchard procedure.[25] The Scatchard Equation expresses the binding of DNA–AO in the presence of K[HL].

$$r_{AO}/c_{AO} = K(n - r_{AO}) \quad (5)$$

where r_{AO} is moles of AO bound per mole of DNA, c_{AO} is the molar concentration of free AO, n is binding site multiplicity per class of binding sites and K is the association binding constant of AO with DNA. Generally, if K[HL] interacts with DNA by the intercalation mode, the value of n remains constant and that of K changes in the Scatchard plot. If K[HL] interacts with DNA by a non-intercalation binding mode involving groove binding or electrostatic interaction, the value of K remains constant and that of n changes in the Scatchard plot. If K[HL] interacts with DNA by a mix binding mode containing non-intercalation and intercalation modes, the values of both n and K change in the Scatchard plot.[26]

Different concentrations of K[HL] ($R_t = c_{K[HL]}/c_{DNA}$ = 0.00, 0.15, 0.30 and 0.45) were used. In order to investigate the effects of electrostatic binding on the interaction between K[HL] and DNA, two groups of experiments with and without the addition of NaCl as a contrast were performed. The influence of NaCl, which is not an anionic quencher of DNA, on K[HL]–AO–DNA comes only from ionic strength.[27] From the Scatchard plots, the values of K and n were obtained. The results are shown in Table I.

TABLE I. Data from the Scatchard Equation for the interaction between K[HL] and DNA

Curve	R_t	NaCl, mass%	Scatchard	K / L mol^{-1}	n
a	0.00	5.00	3.86×10^5–$1.81\times10^7 r_{AO}$	1.81×10^7	0.0212
		0	5.12×10^5–$3.42\times10^7 r_{AO}$	3.42×10^7	0.0145
b	0.15	5.00	5.47×10^5–$2.67\times10^7 r_{AO}$	2.67×10^7	0.0215
		0	3.91×10^5–$2.64\times10^7 r_{AO}$	2.64×10^7	0.0148
c	0.30	5.00	7.55×10^5–$2.60\times10^7 r_{AO}$	2.60×10^7	0.0290
		0	4.30×10^5–$2.43\times10^7 r_{AO}$	2.43×10^7	0.0176
d	0.45	5.00	5.48×10^5–$1.75\times10^7 r_{AO}$	1.75×10^7	0.0303
		0	3.97×10^5–$2.23\times10^7 r_{AO}$	2.23×10^7	0.0178

As can be seen from Table I, the values of both n and K changed with the different concentrations of K[HL]. The variation of the parameters n and K suggested a mixed interaction between K[HL] and DNA. Generally, if n is reduced in the presence of NaCl, electrostatic interaction are indicated,[28] but if n is increased, the existence of groove interactions in the K[HL]–DNA system is indicated. Thus, the results suggest the presence of intercalation and groove binding of K[HL] to DNA.

Viscosity method

In the absence of crystallographic structural data, hydrodynamic measurements, being sensitive to length change, are regarded as the most critical tests for a binding model in solution.[29] To further clarify the interaction between K[HL] and DNA, viscosity measurements were performed.

A classical intercalation mode is known to cause a significant increase in the viscosity of a DNA solution, as the presence of an intercalator forces the effected base-pairs away from each other, thereby causing unwinding the double helix and the lengthening by a given amount of DNA. In contrast, a partial intercalation mode could bend (or kink) the DNA helix and reduce its effective length and, concomitantly, its viscosity. Non-intercalation binding causes no obvious increase in DNA viscosity.[29,30]

The values of the relative specific viscosity $(\eta/\eta_0)^{1/3}$ (where η_0 and η are the specific viscosity contributions of DNA in the absence and in the presence of the K[HL], respectively) were plotted against $c_{K[HL]}$ (Fig. 6). In this study, the relative viscosity of the DNA increased slightly with increasing amounts of the K[HL], but the increase was not as pronounced as those observed for the classical intercalator ethidium bromide.[31] This might be due to the lengthening of the DNA double helix resulting from partial intercalation. This conclusion is in agreement with the other above-mentioned studies.

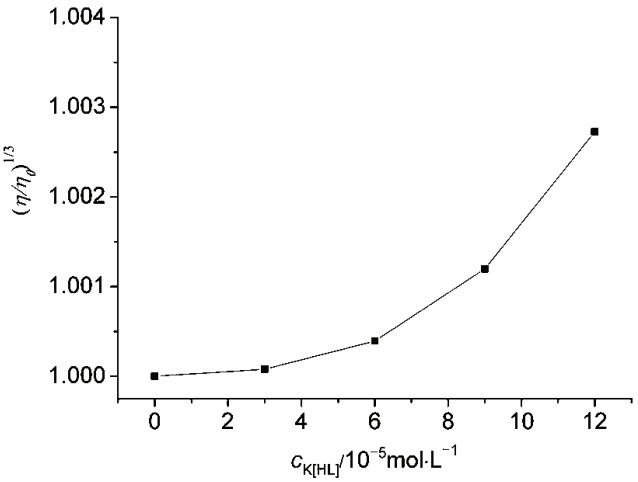


Fig. 6. Effect of increasing amounts of K[HL] on the relative viscosities of DNA at 25 °C; c_{DNA} = = 1.00×10^{-4} mol L^{-1}.

CONCLUSIONS

The Schiff base (K[HL]) derived from vanillin and L-tryptophan was synthesized. The interaction of the Schiff base with hsDNA was studied by UV–Vis absorption, fluorescence and viscosity methods. The obtained results suggest the presence of intercalation and groove binding between K[HL] and DNA. The

internal molecular structure of L-tryptophan, which contains an aromatic indole ring plane, enables K[HL] to insert into the DNA molecule. The information obtained from this work could be helpful to the understanding of the mechanism of the interaction of small molecules with nucleic acids, and should be useful in the development of potential probes of DNA structure and conformation.

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И З В О Д

ИНТЕРАКЦИЈА ИЗМЕЂУ ТРИПТОФАН–ВАНИЛИН ШИФОВЕ БАЗЕ И ДНК ИЗ СПЕРМЕ ХАРИНГЕ

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Интеракција Шифове базе (K[HL]) са ДНК из сперме харинге је проучавана одређивањем UV–Vis апсорпције, флуоресценције и вискозитета у физиолошким условима pH (pH 7,40). Шифова база је синтетисана од ванилина и L-триптофана. Молекули су реаговали у односу $n_{K[HL]}$:n_{DNA} = 5:1. Измерени молски апсорпциони коефицијент, $\varepsilon_{K[HL]\text{-}DNA}$ = 4,98×10^5 L mol^{-1} cm^{-1}, је потврђен методом молских односа. Константе везивања, $K_B^{\ominus}$(301 K) = 1,94×10^5 L mol^{-1} и $K_B^{\ominus}$(310 K) =1,09×10^5 L mol^{-1}, су добијене двоструком реципрочном методом. Термодинамичка мерења указују да је интеракција између K[HL] и ДНК зависна од енталпије. У комбинацији са Скачардовом методом и методом мерења вискозитета, резултати указују на везивање интеркалацијом и преко бразде између K[HL] за DNA.

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