Langmuir Aggregation of Thionin in Nucleic Acids and Its Application

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The microsurface adsorption-spectral correction (MSASC) technique has been described. The formation of the double electrostatic films in nucleic acids causes the monolayer aggregation of stain molecules. The interaction of thionin (TN) with nucleic acids, ctDNA, sDNA and yRNA, has been investigated at pH 7.8. Results show that the binding ratios of TN to ctDNA-P, sDNA-P and yRNA-P are 1.27, 1.30 and 1.40, respectively, the binding constants of the aggregates are 2.8×10^4, 9.10×10^4 and 6.49×10^3 and their molar absorptivities are 1.04×10^4, 1.25×10^4 and 0.94×10^4 $L\cdot mol^{-1}\cdot cm^{-1}$ at 630 nm. The aggregation may be applied to the spectrophotometric detection of nucleic acids samples.

Keywords MSASC technique, nucleic acid, thionin, double electrostatic film, Langmuir aggregation

Introduction

Nucleic acid chemistry has been attracting the attention of many biochemists.[1-7] Understanding the interaction of a chromophore with nucleic acids can help us to realize the function of such genetic biopolymers and analyze the transmission of the genetic information. The quantitative determination of nucleic acids is also significant in clinical tests and laboratory practice. However, the interaction has not been elucidated satisfactorily and earlier observations have not been explained clearly and reasonably, *e.g.*, the Pesavento equation[8] and Scatchard model.[9]

Some chromophores, *e.g.* bromophenol blue and neutral red as the adsorption indicators are usually used in the determination of halogen anions. The same color change was observed when nucleic acid was used in place of halogen anions. Consequently, the interaction of nucleic acid with chromophore has the same mechanism as the precipitation adsorption reaction. Commonly, nucleic acid molecule owns complex spatial structure. The helix, winding and folds lead to many holes, gullies and grooves. The protonated NH_2^+ or NH_3^+ in bases causes the positive electrostatic charge film at the side of the double helix chain and the dehydrophosphate PO_4^- in chain gives a negative electrostatic charge film at the other side (Fig. 1). Thus the double electrostatic films can adsorb small cations and anions, *e.g.* Cl^-, Na^+ and stain ions. Because of the winding and folds of the double helix chain, many microelectrostatic fields will be formed like protein biopolymer.[10] Because this binding depends mainly on the electrostatic force, the adsorption is easy to be destroyed by operation conditions, *e.g.* the addition of high concentration ions (Cl^- and Na^+), higher temperature and so on. The formation of the electrostatic films leads the aggregation and adsorption of small ions or molecules in biopolymers. The aggregation of stain (L) in nucleic acids (M) obey the Langmuir isothermal adsorption.[11] The Langmuir adsorption equation may be used:

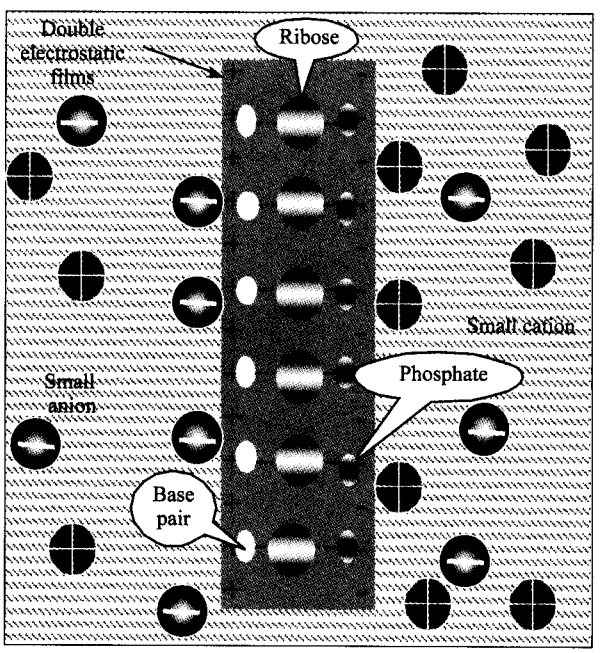

Fig. 1 Formation of the double electrostatic film on the double helix chain of nucleic acid and adsorption of stain ions on its surface.

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Received May 10, 2002; revised and acceptted October 28, 2002.

Project supported by the Natural Science Foundation of Anhui Province (No. 01045301) and the Anhui Provincial Science and Technology Foundation for Excellent Youths.

$$\frac{1}{\gamma} = \frac{1}{N} + \frac{1}{NKc_L} \quad (1)$$

where K is the binding constant and c_L is the molar concentration of excess of L. N indicates the maximal binding ratio of L to M and γ is the molar ratio of L adsorbed to M. K is calculated from Eq. 1. Both c_L and γ are calculated by means of:[12-14]

$$\gamma = \eta \times \frac{c_{L0}}{c_M} \quad (2)$$

$$c_L = (1 - \eta) c_{L0} \quad (3)$$

$$\eta = \frac{A_c - \Delta A}{A_0} \quad (4)$$

where both c_M and c_{L0} are the concentration of the M and L added initially and η indicates the effective fraction of L. A_c, A_0 and ΔA are the real absorbance of the M-L product, the measurement absorbance of the reagent blank against water and that of the M-L solution against reagent blank directly measured at the peak wavelength λ_2. Within increase in L molar concentration, γ approaches a maximum at N. The A_c is calculated by means of:[15,16]

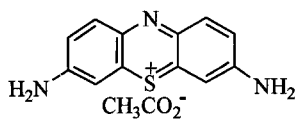
$$A_c = \frac{\Delta A_c - \beta \Delta A'}{1 - \alpha\beta} \quad (5)$$

where the symbol $\Delta A'$ indicates the absorbance of the M-L solution measured respectively at the valley absorption wavelength λ_1. Usually, α and β are the correction constants[13] and they are calculated by measuring directly ML_N and L solutions. In addition, the real molar absorptivity (real $\varepsilon_r^{\lambda_2}$ not apparent $\varepsilon_a^{\lambda_2}$ of the aggregate ML_N can be calculated by the means of:

$$\varepsilon_r^{\lambda_2} = \frac{NA_c}{\delta \gamma c_M} \quad (6)$$

where the symbol δ is the cell thickness (cm) and the others have the same meanings as in the equations above.

The Pesavento hypothesis and Scatchard model are similar to the Langmuir adsorption Eq. (1). Consequently, both the Pesavento hypothesis and Scatchard model are just the adsorption of microelectrostatic field between biopolymers and small molecules. The cooperation of both the Langmuir adsorption and the spectral correction technique provides a very helpful experimental strategy for study of aggregation of chromophore in biopolymers. The approach is called microsurface adsorption-spectral correction technique (MSASC). In this work, the interaction of thionin (TN) with nucleic acids was investigated. The structure of the chromophore is given as below:



Thioin (TN)

It forms cations at neutral medium and is able to be strongly adsorbed on the negative electrostatic film of the double helix of nucleic acid. The aggregation of TN in nucleic acids (ctDNA, sDNA and yRNA) is sensitive. The aggregation obeys the Langmuir isothermal adsorption in only monolayer. The characterization of the TN-nucleic acid aggregates was made and the aggregation was tried to apply to the detection of nucleic acid samples.

Experimental

Materials and methods

Absorption spectra were recorded with a TU1901 spectrophotometer (PGeneral, Beijing). DDS-11A conductivity meter (Tianjin Second Analytical Instruments) was used to measure conductivity together with a DJS-1 conductivity immersion electrode (electrode constant 0.98) (Shanghai Tienkuang Devices) in the production of deionized water of 0.5—1 $\mu\Omega^{-1} \cdot cm^{-1}$. The pH of the solution was measured with a pHS-2C acidity meter (Leici Instruments, Shanghai) and Model 620D pH Pen (Shanghai Ren's Electrics). The temperature was adjusted and remained constant in a Model 116R electric heated thermostatic bath (Changjiang Test Instruments of Tongjiang, China).

Calf thymus (ct) DNA and salmin (s) DNA were purchased from Sigma Chemicals and yeast (y) RNA from Shanghai Chemical Reagents of Chinese Medicine Group. According to a standard procedure previously described,[4] they were stored in 1 $mmol \cdot L^{-1}$ phosphate buffer (pH 7, containing 10 $mmol \cdot L^{-1}$ NaCl). The molar concentrations of ctDNA (0.612 mg/mL in weight concentration), sDNA (0.506 mg/mL) and yRNA (0.431 mg/mL) were obtained via absorbance measurement using $\varepsilon_{ctDNA} = 6600\ L \cdot mol^{-1} \cdot cm^{-1}$ and $\varepsilon_{yRNA} = 7800\ L \cdot mol^{-1} \cdot cm^{-1}$ at the maximum near 260 nm (*i.e.*, nucleic acid concentrations are reported in molar base pairs).[17] TN solution (0.420 $mmol \cdot L^{-1}$) was prepared by dissolving 0.0672 g of TN (content 90%, Chroma) in 10 mL of DMF and diluted to 500 mL with deionized water. The Britton-Robinson buffer solutions between pH 2.5 and 11.7 were prepared to control the acidity of the interaction solution. 2 $mol \cdot L^{-1}$ NaCl was used to adjust the ionic strength of the aqueous solutions. Na_2EDTA solution (1%) was prepared to mask the foreign metallic ions co-existing possibly in the practical samples.

Into a 10 mL calibrated flask were added an appropriate working solution of nucleic acids, 1.0 mL of Britton-Robinson buffer solution and a known volume of TN solution. The mixture was then diluted to 10 mL with deionized water and mixed thoroughly. After 10 min, the absorption measurement was made against the blank treated in the same way without nucleic acids.

In the quantitative determination of nucleic acids in samples, the masking reagent, 0.5 mL of Na_2EDTA solution (1%) was added to complex metal ions, *e.g.* Pb(II), Cd(II), Hg(II) and so on. The next successive operation is

the same as the above procedure.

Results and discussion

Spectral analysis

The adsorption of TN in nucleic acids (ctDNA as representative) was carried out. At pH 7.8, the absorption spectra of the ctDNA-TN solutions are shown in Fig. 2. From curve 1, the peak of TN is located at 592 nm. From curve 2, the absorbance ratio of the solutions reaches a minimum and then almost remains constant when the molar ratio of ctDNA-P to TN is more than 5. So there is no free TN in solutions containing over 1.5 mg of ctDNA (equal to about 4.5 μmol DNA-P) and 0.840 μmol of TN. Curve 3 gives the spectrum of such a solution. The peak of the ctDNA-TN aggregate is located at 610 nm. By comparing curve 1 with curve 3, the spectral red shift of the aggregate is only 18 nm. Curves 4 and 5 give the relative spectra of the ctDNA-TN solution against TN solution without ctDNA. The use of pH 7.8 buffer solution gives the higher peak and valley than that of pH 3.8 buffer solution. From curve 4, the peak is located at 592 nm and the valley at 623 nm. So, such two wavelengths were used in this study. From curves 1 and 3, the correction coefficients were calculated to be $\beta = 0.174$ and $\alpha = 0.964$. So $A_c = 1.20\ (\Delta A - 0.174\Delta A')$.

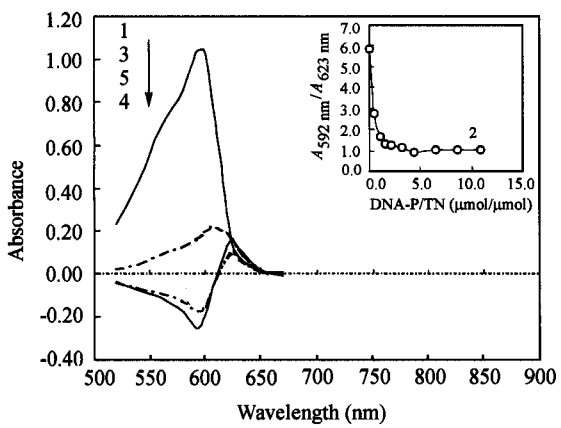


Fig. 2 Absorption spectra of TN and its ctDNA solutions at pH 7.8. (1) 1.26 μmol of TN at pH 7.8; (2) variation of the two absorbance ratio of solution at 592 and 623 nm with molar ratio of ctDNA-P to TN at pH 7.8; (3) solution initially containing 0.420 μmol of TN and 1 mg of ctDNA at pH 7.8; (4) solution initially containing 1.26 μmol of TN and 0.306 mg of ctDNA at pH 7.8 and (5) the same as (4) but at pH 3.8. Both (4) and (5) against reagent blank and the others against water.

Effect of pH, *ionic strength and temperature on aggregation*

By varying pH of solution between 2.5 and 11.5, the absorbance of the ctDNA-TN solution was measured and γ of TN to ctDNA-P is shown in Fig. 3. The aggregation is sensitive in a wide pH scope. This is attributed to the fact that TN can form cations no matter where it is in acidic or basic aqueous solution. TN cation is attracted easily and closely on the groove surface with negative charges in nucleic acids.

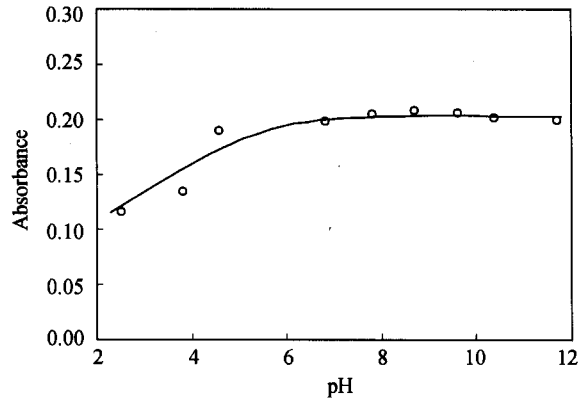


Fig. 3 Effect of pH on A_c of solution initially containing 0.840 μmol of TN and 0.306 mg of ctDNA.

The influence of ionic strength of solution on γ is shown in Fig. 4a. γ decreases notably with increase in ionic strength between the ionic strength of 0 and 1 $mol \cdot L^{-1}$. This is attributed to the fact that more inorganic ions were attracted on DNA to take up the double electrostatic films of the grooves of DNA.

Between 16 and 80 ℃, variation of γ of TN to ctDNA is shown in Fig. 4b. γ decreases by about 25% per increasing 10 ℃. The electrostatic attraction is often weaker than chemical bond, so the adsorption is easily destroyed by a high temperature. Interestingly, the increase of γ was observed when the temperature of the solution reduced from 80 ℃. Therefore, the aggregation of TN in biopolymer is reversible but the final γ is often less than the initial one.

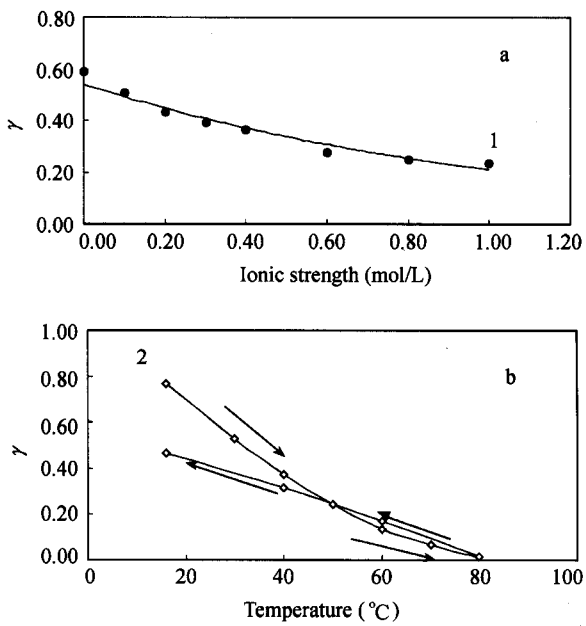


Fig. 4 Effect of ionic strength (a) and temperature (b) on γ of solution initially containing: (1) 0.840 μmol of TN and 0.306 mg of ctDNA and (2) 1.26 μmol of TN and 0.306 mg of ctDNA, respectively.

Effect of TN *on aggregation*

By varying the addition of TN solution, the absorption of solutions was measured. γ and c_L of each solution were calculated. Their relationships are shown in Fig. 5. All are linear so the aggregation of TN in nucleic acids obeys the Langmuir isothermal adsorption. From the intercepts of lines, the maximal binding ratio, N of TN to ctDNA-P, sDNA-P and yRNA-P are calculated to be 1.27:1, 1.30:1 and 1.40:1, respectively. From the slopes, the binding constants of the aggregates were calculated to be 2.80×10^4, 9.10×10^4 and 6.49×10^3 L·mol^{-1} and their real absorptivities to be 1.04×10^4, 1.25×10^4 and 0.94×10^4 L·mol^{-1}·cm^{-1} at 630 nm. In characterization of an aggregate, the spectral correction method has some special advantages in operation and principle by contrast of the classical methods such as molar ratios,[18] continuous variations[19] and equilibrium movements.[20]

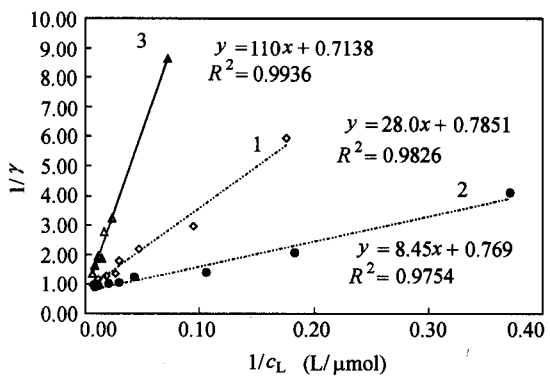


Fig. 5 Plots γ^{-1} vs. c_L^{-1}. Solutions initially containing (1) 0.306 mg of ctDNA, (2) 0.253 mg of sDNA and (3) 0.215 mg of yRNA.

Fig. 6 gives out the effect of various additions of 0.420 mmol·L^{-1} TN on A_c of the TN-nucleic acid solutions and η of TN. From curves 1-1, 1-2 and 1-3, A_c remains almost maximal and constant at over 4.0 mL of 0.420 mmol·L^{-1} TN. From curves 2-1, 2-2 and 2-3, at the addition of 5.0 mL of TN solution, the effective TN is only 42% in the solution containing 0.306 mg of ctDNA, 45% in the solution containing 0.253 mg of sDNA and 22% in the solution containing 0.215 mg of yRNA. Therefore, all over half of TN will be excessive and free in such solutions. It will affect notably the measurement of absorbance of the aggregate. So the spectral correction technique was used in place of ordinary spectrophotometry.

Application

The standard series of nucleic acids were prepared and measured at pH 7.8 where 5.0 mL of 0.420 mmol·L^{-1} TN was added. The results are shown in Fig. 7. From these slopes of the regression equations, each slope by the spectral correction method is always more than that by ordinary spectrophotometry. So the spectral correction method has a higher sensitivity than ordinary one. By adding Na_2EDTA solution (0.5 mL of 1%) in nucleic acid solution, the influence of foreign substances including ions and organic compounds, on the determination of nucleic acid was tested at pH 7.8. None of the following ions affected the direct determination of 0.306 mg of ctDNA (less than 10% error): 0.5 mg of SO_4^{2-}, Cl^-, NO_3^-, SO_3^{2-}, $C_2O_4^{2-}$, Ac^-, BSA, glucose, amino acid, ethanol, Ca(II) and Mg(II), 0.2 mg of NH_4^+, F^-, PO_4^{3-}, Mn(II), Zn(II) and Pb(II), 0.05 mg of Fe(III), Ni(II), Co(II), Cd(II) and Cu(II). The anionic surfactants, SDS and SDBS brought the determination negative error.

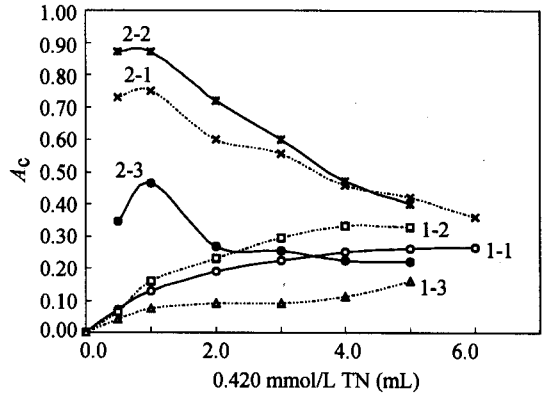


Fig. 6 Effect of TN on $A_c(1\text{-}x)$ and η $(2\text{-}x)$ of solutions initially containing $(x\text{-}1)$ 0.306 mg of ctDNA, $(x\text{-}2)$ 0.253 mg of sDNA and $(x\text{-}3)$ 0.215 mg of yRNA.

Two samples were determined. The first sample was prepared in Children Drink background and the other was prepared with drinking water background. To them, were added drops of nucleic acid and the following compounds or ions: 1 mg of K^+, Ca(II), glucose and PO_4^{3-}, 0.5 mg of Mg(II), F^- and 0.1 mg of Fe(II), Cu(II), Pb(II) and Zn(II). The recovery is between 91.5% and 105% and the relative standard deviation is less than 4.3%.

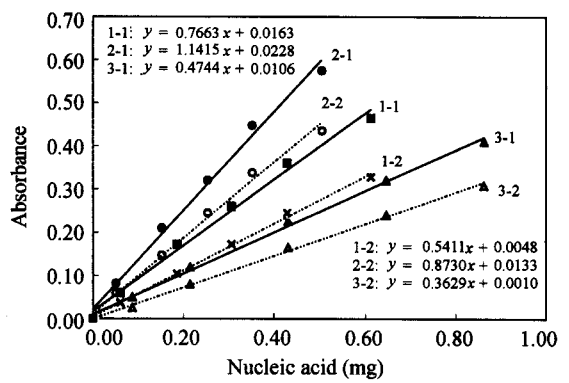


Fig. 7 Standard curves for determination of nucleic acids using TN as probe reagent at pH 7.8 in the presence of EDTA. (1-1) ΔA_c of ctDNA-TN aggregate and (1-2) ΔA of ctDNA-TN solution at 623 nm, (2-1) A_c of sDNA-TN aggregate and (2-2) ΔA of sDNA-TN solution at 623 nm, (3-1) A_c of yRNA-TN aggregate and (3-2) ΔA of yRNA-TN solution at 623 nm.

Conclusion

The investigation to the interaction of TN with nucleic acids supports the Langmuir monolayer adsorption of a stain in biopolymer. Though MSASC technique has not given the higher sensitivity than other methods such as RLS,[3] it meets precision and accuracy criteria and offers the additional benefits of simplicity and versatility.

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(E0205101 LU, Y. J.)