

Langmuir Aggregation of Dahliaviolett on DNA

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Formation of double electrostatic films in DNA causes Langmuir aggregation of small ions and molecules only in a monolayer. We studied the interaction of dahliaviolett (DLV) with DNA at pH = 7.24 by a spectral correction technique. Results showed that the adsorption ratio of DLV to DNA-P was 1 : 4, the adsorption constant of the DLV-DNA aggregate was 4.44×10^4 and its molar absorption coefficient was $1.02 \times 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 615 nm. This aggregation was applied to the quantitative determination of DNA in samples.

Key words: DNA, dahliaviolett, double electrostatic films, Langmuir aggregation, spectral correction technique.

INTRODUCTION

Macromolecules and biopolymers are always very actively studied^{1–7} by spectroscopic techniques, such as spectrophotometry, fluorimetry, Rayleigh-light scattering (RLS), *etc.* Nucleic acid chemistry belongs to an advanced field and is attracting more and more biochemists to research. The use of probe, *e.g.* stain in macromolecular solution, helps to understand the interaction of small ions or molecules with macromolecules, *e.g.* protein and nucleic acid, and to analyze the transmission of genetic information. However, the interaction has not been elucidated satisfactorily and earlier observations have not been explained clearly and reasonably, *e.g.* the Pesavento equation⁸ and the Scatchard model.⁹

DNA has a complex spatial structure. The helix, winding and folds form many holes, gullies and grooves. Protonation of $-\text{NH}_2$ on the base pair to

form NH_3^+ causes the positively charged film on a side of the double helix chain and the dehydrophosphate of HPO_4^- on two main chains to form PO_4^- , providing a negatively charged film on the other side (Figure 1). Double electrostatic films can adsorb small cations and anions, *e.g.* Cl^- , Na^+ , stain ions like protein.¹⁰ As this binding depends mainly on the electrostatic force, the adsorption is easily destroyed by operation conditions, *e.g.* addition of high concentrations of ions (*e.g.* Cl^- , Na^+), higher temperature, and

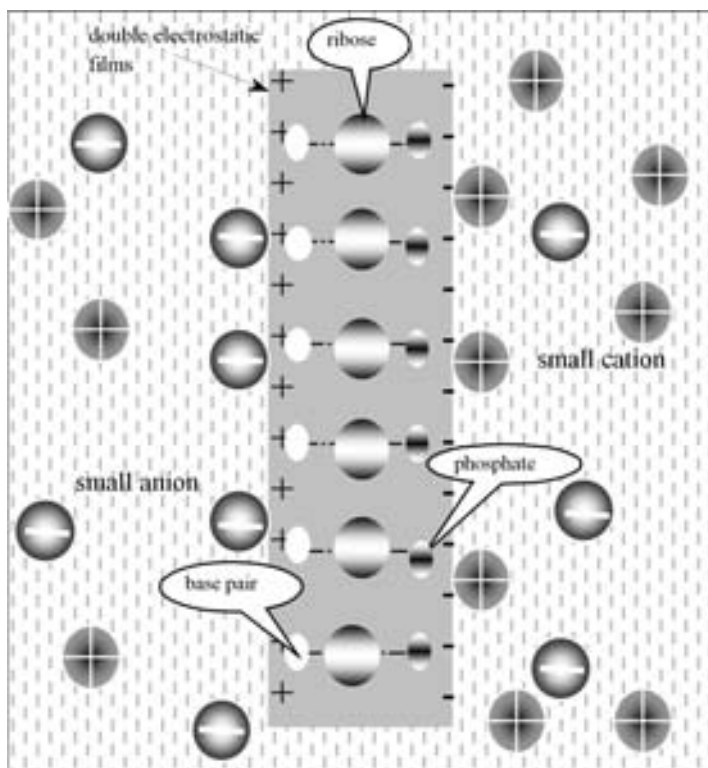


Figure 1. Formation of double electrostatic films in the helix grooves of DNA and adsorption of stain ions.

so on. The formation of electrostatic films leads to aggregation of stains in biopolymers. The aggregation of stains (L) in DNA obeys the Langmuir isotherm adsorption.¹¹ The adsorption relation is:

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

where K indicates the adsorption constant of the stain-biomacromolecule aggregate and c_L is the concentration (molarity) of free L in a M-L (M, biomacromolecule) equilibrium solution. Symbol N indicates the maximal adsorption ratio of L to M and γ is the molar ratio of the effective L adsorbed on M. Both K and N are calculated from Eq. (1) Both c_L and γ are calculated by means of equations:¹²

$$\gamma = \eta \times \frac{c_{L_0}}{c_M} \quad (2)$$

$$c_L = (1 - \eta)c_{L_0} \quad (3)$$

where

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

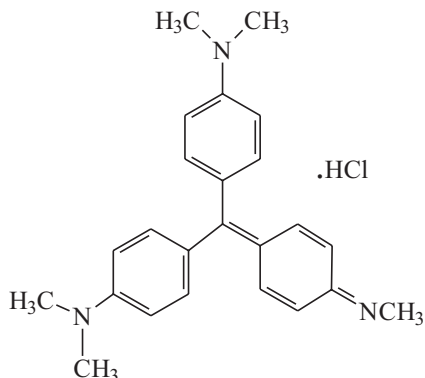
$$A_c = \frac{\Delta A - \beta \Delta A'}{1 - \alpha \beta} \quad (5)$$

where both c_M and c_{L_0} are the initial molar concentrations of M and L and η indicates the effective fraction of L adsorbed. Symbols A_c and A_0 are the real absorbance of the M-L aggregate, which was calculated by Eq. (5) and the absorbance of a reagent blank against water measured at the peak wavelength (λ_2). Symbols ΔA and $\Delta A'$ are the absorbance of the M-L solution against a reagent blank measured at λ_2 and one at the valley wavelength (λ_1). With an increase in L concentration, γ will approach a maximum at N . In the above equations, α and β are the correction constants,¹³ and they can be calculated by directly measuring ML_N and L solutions. In addition, the molar absorption coefficient (real $\epsilon_r^{\lambda_2}$ not apparent $\epsilon_a^{\lambda_2}$) of aggregate ML_N at λ_2 is also directly calculated by means of the expression:

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

where δ is the cell thickness (δ/cm) and the other symbols have the same meanings as in the above equations.

We found that the Pesavento hypothesis and the Scatchard model are the same as the Langmuir isothermal equation (1). Combination of the Langmuir adsorption and the spectral correction technique (MPASC) provides a very helpful experimental strategy for studying the aggregation of a stain on a biopolymer. In the present work, we studied the interaction of dahliaviolett (DLV) with DNA. The structure of DLV (Methyl Violet 2B, C.I. No. 4253) is given below:



It can dechlorinate to form cations in a neutral medium, which may be adsorbed on the negatively charged film of DNA. We found that the aggregation of DLV in DNA is sensitive and it was applied to the quantitative determination of DNA in samples.

EXPERIMENTAL

Apparatus and Reagents

The absorption spectra were recorded with a TU1901 spectrophotometer (PGeneral, Beijing), the independent absorbance of solution was measured with a Model 722 (Shanghai 2nd Analytical Instruments). A Model DDS-11A conductivity meter (Tianjin Second Analytical Instruments) was used to measure the conductivity together with a Model DJS-1 conductivity immersion electrode (electrode constant 0.98) (Shanghai Tienkuang Devices) for the production of deionized water of $0.5\text{--}1\ \mu\Omega^{-1}\ \text{cm}^{-1}$. The pH of a solution was measured with a PHS-2C acidity meter (Leici Instruments, Shanghai, China) and a Model 620D pH Pen (Shanghai Ren's Electronics). The temperature was adjusted and kept constant in a Model 116R electrically heated thermostatic bath (Changjiang Test Instruments of Tongjiang, China).

Calf thymus (ct) DNA was purchased from Sigma Chemicals. Using a standard, previously described procedure⁴ it was stored in $1\ \text{mmol}\ \text{dm}^{-3}$ phosphate buffer, pH = 7, containing $10\ \text{mmol}\ \text{dm}^{-3}$ NaCl. The molar concentrations of DNA were obtained *via* absorbance measurements using $\epsilon_{\text{DNA}} = 6600$ at $260\ \text{nm}$ (*i.e.*, DNA concentrations are reported in molar base pairs).¹⁴ The DLV solution ($0.235\ \text{mmol}\ \text{dm}^{-3}$) was prepared by dissolving $0.1090\ \text{g}$ of DLV (made in Schmiel GmbH) in $1000\ \text{ml}$ of deionized water. The neutral Britton-Robinson buffer solution of pH = 7.24 was prepared to control the acidity of the interaction solution to keep it near to that of a living organism. $2\ \text{mol}\ \text{dm}^{-3}$ NaCl was used to adjust the ionic strength of the aqueous solutions. Na_2edta solution (1% in deionized water) was prepared to mask any foreign metal ions that might have co-existed in the samples.

Methods

Into a 10 ml calibrated flask, an appropriate working solution of DNA, 1.0 ml of Britton-Robinson buffer solution and a known volume of DLV solution were added. The mixture was then diluted to 10 ml with deionized water and mixed thoroughly. After 10 min, the absorption measurement was made at 585 and 615 nm, against the blank treated in the same way without DNA.

In the quantitative determination of DNA in the samples, 0.5 ml of 1% Na₂edta was added to complex metal ions and the subsequent procedures were the same as those mentioned in the last paragraph.

RESULTS AND DISCUSSION

Spectral Analysis

Adsorption of DLV on DNA was done at pH = 7.24, and their absorption spectra are shown in Figure 2. From curve 1, the peak of DLV is located at 585 nm. From curve 2, the absorbance ratio of DLV to DNA approaches a minimum when the ratio of DNA-P to DLV is more than 60. Thus, no free DLV is contained in such a solution. Curve 3 shows its spectrum. From

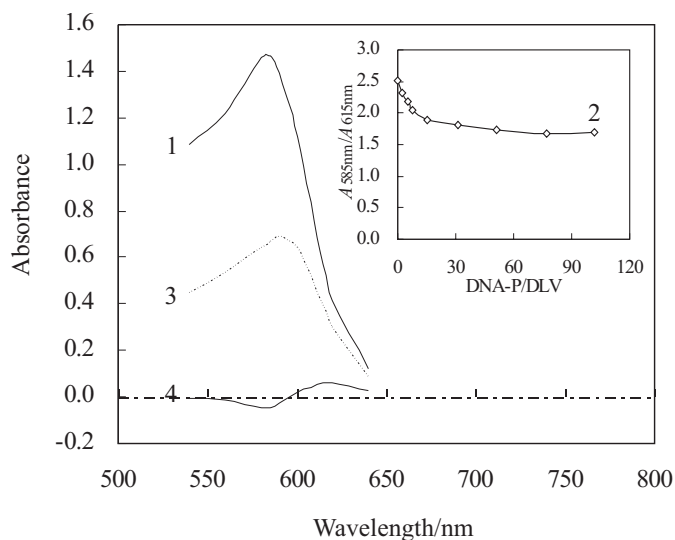


Figure 2. Absorption spectra of DLV and its DNA solutions at pH = 7.24: 1) 0.0235 $\mu\text{mol ml}^{-1}$ DLV, 2) variation of the ratio of absorbance measured at 585 nm to that measured at 615 nm of the DLV-DNA solutions with different molar ratio DNA-P/DLV, 3) 0.0117 $\mu\text{mol ml}^{-1}$ DLV plus 0.900 $\mu\text{mol ml}^{-1}$ DNA-P, 4) 0.0352 $\mu\text{mol ml}^{-1}$ DLV plus 0.050 mg ml^{-1} DNA. Only 4 against a reagent blank and the others against water.

curve 3, the peak of the DNA-DLV aggregate is located at 610 nm. By comparing curves 1 and 3, the spectral red shift of the aggregate is only 18 nm. Curve 4 shows the relative spectrum of the DNA-DLV solution. From it, we observe that the peak is located at 615 nm and the valley is at 585 nm. These two wavelengths were used in this study. From curves 1 and 3, the correction coefficients were calculated to be $\beta = 0.388$ and $\alpha = 1.69$. So $A_c = 2.90 (\Delta A - 0.388\Delta A')$.

Effect of DLV Concentration on DLV Aggregation

By varying addition of the DLV solution, absorptions of the DLV-DNA solutions were measured. The values of γ and c_L were calculated. Their relationship is shown in Figures 3 and 4. From curve 2, A_c approaches a maximum when DLV concentration is over $0.0235 \mu\text{mol ml}^{-1}$. From curve 3, with addition of 1.0 ml of the DLV solution, the effective DLV is only 50%. Therefore, a half of the DLV added is excessive and free. Beyond all doubt, it will interfere notably with the absorbance measurement of the aggregate. The spectral correction technique was used here instead of ordinary spectrophotometry. From Figure 4, the relationship γ^{-1} vs. c_L^{-1} is linear, so the aggregation of DLV on DNA obeys the Langmuir isothermal adsorption only in a monolayer. From the intercept, the maximal binding ratio (N) of DLV to DNA-P is calculated to be about 0.25. This indicates that 4 phosphates in

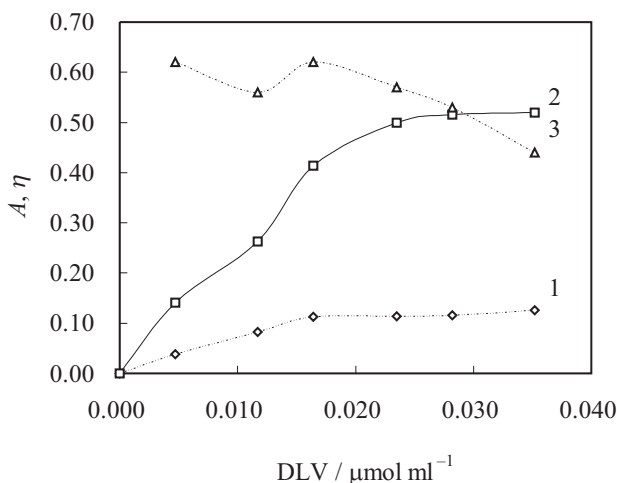


Figure 3. Effect of DLV concentration on the absorbance, A ; and the effective fraction of DLV adsorbed, η : 1) ΔA measured at 615 nm, 2) A_c measured at 615 nm, 3) η of DLV.

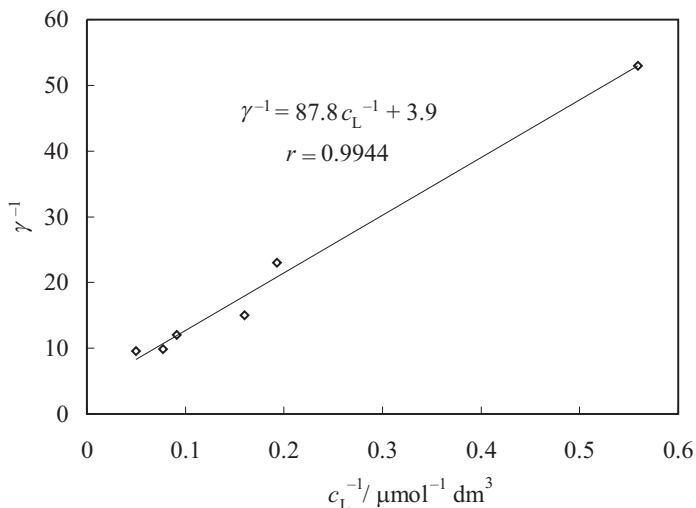
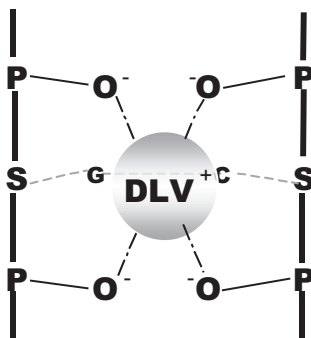


Figure 4. Relation γ^{-1} vs. c_L^{-1} . γ , molar ratio of the effective amount of L (adsorbed on M) and M; c_L , concentration of free L in the M-L equilibrium solution.

DNA can adsorb one DLV molecule with the electrostatic force. The following diagrammatic sketch is suggested:



One turn of the double helix of DNA will bind 5 DLV molecules. From the slope, the adsorption constant (K) of the aggregate was calculated to be 4.44×10^4 . Its molar absorption coefficient (ϵ) was calculated to be $1.02 \times 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 615 nm by Eq. (6). In the determination of a binding ratio and an adsorption constant, the spectral correction technique has special advantages compared to the classical methods such as the molar ratios and continuous variations.

Effect of Ionic Strength and Temperature on DLV Aggregation

The influence of ionic strength on the binding ratio of DLV to DNA-P is shown in Figure 5. From curve 1, we observed that the binding ratio of DLV to DNA-P greatly decreases with an increase in the ionic strength. This is attributed to the fact that a high concentration of Na^+ can take place of DLV to take up the negatively charged film of DNA. This causes a notable reduction of the binding ratio of DLV to DNA-P.

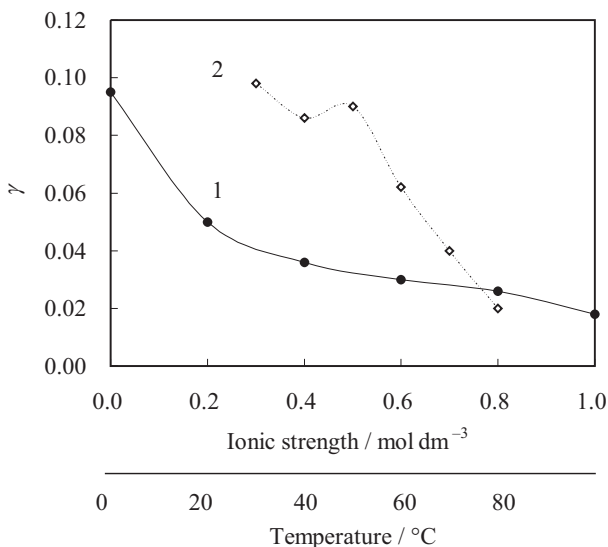


Figure 5. Effect of ionic strength (1) and temperature (2) on the binding ratio of DLV ($0.0235 \mu\text{mol ml}^{-1}$) to DNA (0.020 mg ml^{-1}) at $\text{pH} = 7.24$.

Between 30 and 80 °C, the variation of the adsorption ratio of DLV to DNA-P is shown in Figure 5, as well. From curve 2, we observed that the adsorption ratio decreases along with an increase in temperature. The adsorption ratio decreases by 30% with a temperature increase of 10 °C. This is attributed to the fact that a high temperature can result in a rapid desorption of DLV from DNA. In addition, the electrostatic attraction of a stain on DNA is often much weaker than a chemical bond. Such an adsorption is easily destroyed by a higher temperature.

Quantitative Determination of DNA

A standard series of DNA was prepared and measured at $\text{pH} = 7.24$ when 1.0 ml of $0.235 \text{ mmol dm}^{-3}$ DLV was added. Between 0 and 0.080 mg

ml⁻¹ DNA, the linear regression equation is $A_c = 9.63x + 0.011$ ($x/\text{mg ml}^{-1}$; $r = 0.9944$). By adding the Na₂edta solution, none of the following ions affected direct determination of 0.020 mg ml⁻¹ DNA (less than 10% error): 0.1 mg ml⁻¹ protein, glucose, amino acid, Ca^{II}, Mg^{II}, F⁻, PO₄³⁻, 0.01 mg ml⁻¹ Mn^{II}, Zn^{II}, 0.005 mg ml⁻¹ Ni^{II}, Pb^{II}, Cu^{II}. The anionic surfactants SDS and SDBS can bring about a positive error.

Determination of DNA in Samples

Two samples were determined. The first sample was prepared with a Children Drink background and the other was prepared with a drinking water background. Into them, drops of DNA solution and the following compounds or ions were added: 0.1 mg ml⁻¹ K⁺, Ca^{II}, glucose and PO₄³⁻, 0.05 mg ml⁻¹ Mg^{II}, F⁻ and 0.01 mg ml⁻¹ Fe^{II}, Cu^{II}, Pb^{II} and Zn^{II}. The analysis of the two samples showed the recovery of DNA between 91.2 and 103%, and the RSDs of less than 4.3%.

CONCLUSION

This investigation of the interaction of DLV with DNA supports the Langmuir isothermal adsorption of small molecules in a biomacromolecule. Though the MPASC technique has not given higher sensitivity than other methods, such as RLS,³ it meets the precision and accuracy criteria and offers additional benefits of simplicity and versatility. We maintain that classical spectrophotometry can still play an important role in explaining the macromolecular behavior.

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SAŽETAK

Langmuirova agregacija bojila »dalija-ljubičasto« s DNA

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Nastajanje električkog dvosloja oko molekule DNA ima za posljedicu Longmuirovu agregaciju malih iona i molekula u monosloju. Spektroskopski je proučena interakcija bojila »dalija-ljubičasto« (DLV) s DNA pri pH = 7. Rezultati pokazuju da je adsorpcijski omjer DLV/DNA-P 1:4, da adsorpcijska konstanta DLV-DNA agregata iznosi $4,44 \times 10^4$ te da je molarni adsorpcijski koeficijent pri 615 nm $1,02 \times 10^5$ L mol⁻¹ cm⁻¹. Agregacija je primijenjena za kvantitativno određivanje DNA u uzorcima.