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Interaction Between Humic Acid and DNA

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Abstract

Humic acid (HA) are widely dispersed, naturally occurring biopolymers most commonly found in soil, drinking water, and plants. Humic acid is soluble in alkali and insoluble in an acidic medium or water. In the present study the interaction of Humic acid with DNA is determined by the calculation of the binding constant of humic acid with CT-DNA and the quenching phenomenon of humic acid in the presence of DNA is also studied using Stern Volmer Relationship.

Experimental Method:

Reagents and Materials

- Sigma Aldrich Humic acid Lot # BCBC9785V
- Rockland DNA (Calf Thymus) 100mg Lot # 24420
- 523 µM CT-DNA prepared from stock solution.

Instruments and Equipment

The instruments used for this study were

Perkin Elmer Lambda 35 UV-Vis Spectrophotometer at room temperature

Ocean Spectra USB 2000 Fluorometer at room temperature

Humic acid Stock solution:

Humic acid is said to be insoluble in water. The solubility of humic acid in water is determined by a pH dependent study of the solubility. As humic acid is soluble in alkali solutions the pH of the water was maintained at alkaline range using a few drops 0.5M NaOH solution. The pH of the water is monitored using pH meter and known amount of humic acid was added to water to check the solubility of the solution and then the pH of the solution is increased by titrating with

0.5M NaOH till the Humic acid was completely dissolved in water. The Humic acid was dissolved in water at a pH of 9.9.Then 0.013 g of HA was dissolved in 10ml of water which ai maintained at a pH of 9.9 and slowly the water (at pH 9.9) is added to the Humic acid solution till the HA is completely dissolved. The known amount of HA (0.013g) was dissolved in 160ml of water at pH 9.9.The concentration of the stock solution was calculated to be 358 µM. The prepared HA stock solution is used for further study.

DNA Binding Studies:

The spectrophotometric method is used to calculate the DNA binding constant of Humic Acid. A series of ten different solutions were prepared. In each vial 1.5ml of HA is added and the DNA concentration was increased from 0.1-1.0 ml and the final volume was made up to 3 ml with distilled water.

Table 1. Sample solutions for UV spectroscopic determination

S.No	Sample
Vial 1	1.5ml of HA+ 0.1ml of DNA+ 1.4ml of water
Vial 2	1.5ml of HA+ 0.2ml of DNA+ 1.3ml of water
Vial 3	1.5ml of HA+ 0.3ml of DNA+ 1.2ml of water
Vial 4	1.5ml of HA+ 0.4ml of DNA+ 1.1ml of water
Vial 5	1.5ml of HA+ 0.5ml of DNA+ 1.0ml of water
Vial 6	1.5ml of HA+ 0.6ml of DNA+ 0.9ml of water
Vial 7	1.5ml of HA+ 0.7ml of DNA+ 0.8ml of water
Vial 8	1.5ml of HA+ 0.8ml of DNA+ 0.7ml of water
Vial 9	1.5ml of HA+ 0.9ml of DNA+ 0.6ml of water
Vial 10	1.5ml of HA+ 1.0ml of DNA+ 0.5ml of water

The absorbance spectra of the above prepared solution were measured at 450 nm. The absorbance values were used to calculate the molar absorptivity of the samples using Beer-Lamberts law.

$$A= \varepsilon cl$$

Where ε= Molar absorptivity of the sample

A= absorbance of the sample at a given wavelength

c= concentration of the drug

l= path length

The concentration of DNA in each vial is calculated using the formula

$$M_1V_1=M_2V_2$$

Where M_1= initial concentration of DNA

V_1= volume of DNA added

M_2= final concentration of DNA

V_2= final volume of solution in the vial.

Table 2: Calculated values of molar absorptivity and concentration of DNA.

Vial	Molar extinction coeff (ε) ($M^{-1}cm^{-1}$)	DNA conc (μM)	1/[DNA] (μM^{-1})
1	0.00645	17.43	0.0573
2	0.00426	34.86	0.0286
3	0.00449	52.3	0.0191
4	0.00353	69.73	0.0143
5	0.00294	87.16	0.0114
6	0.00245	104.6	0.0095
7	0.00242	122.1	0.0081
8	0.00227	139.4	0.0071
9	0.00211	156.8	0.0063
10	0.00208	174.3	0.0057
Humic acid	0.00082	0	0

Optical Titration Method:

The DNA binding constant of a compound is determined by using optical titration method. The binding constant (K_b) was calculated using the following equation.

$$(\varepsilon_a-\varepsilon_b)/(\varepsilon_b-\varepsilon_f)= (1/K_b)x(1/[DNA])+1$$

Where ε_a = molar extinction coefficient of each vial

ε_b= molar extinction coefficient of vial 10

ε_f = molar extinction coefficient of humic acid

By using the above equation $(\varepsilon_a-\varepsilon_b)/(\varepsilon_b-\varepsilon_f)$ values were calculated for all the 10 different vials. The binding constant of each molecule was determined by plotting $(\varepsilon_a-\varepsilon_b)/(\varepsilon_b-\varepsilon_f)$ vs 1/[DNA], and Kb was calculated as the reciprocal value of the slope.

Stern-Volmer Equation:

Quenching is a process that decreases the fluorescence intensity. Collisional quenching is described by Stern Volmer Equation.

$$\frac{Fo}{F} = 1 + Kq\,[Q]$$

Where F_0= Fluorescence intensity without the quencher

F= Fluorescence intensity with the quencher

Kq= Quenching rate constant

[Q]= concentration of the quencher

The term Kq is the second order rate constant that describes the quenching process. It is proportional to the effectiveness of the quencher and the accessibility of the fluorophore to the collisions with the quencher.

Determination of Kq using Stern Volmer Plot:

To determine the quencher rate constant 3ml (358μM) of Humic acid stock solution was taken in a fluorescence cuvette and the maximum intensity was observed at 530 nm. Then CT-DNA was added to the above solution in 20 μl increments for upto 10 readings. The quencher rate constant (Kq) is determined from the slope of the line for the graph of intensities vs concentration of the quencher.

Table 3: Fluorescence intensities of the solutions

Sample	Intensity @ 530nm
3ml of HA	266.33
3ml of HA+ 20µl DNA	262.96
3ml of HA+ 40µl DNA	262.57
3ml of HA+ 60µl DNA	262.08
3ml of HA+ 80µl DNA	261.38
3ml of HA+ 100µl DNA	261.22
3ml of HA+ 120µl DNA	257.37
3ml of HA+ 140µl DNA	257.19
3ml of HA+ 160µl DNA	255.13
3ml of HA+ 200µl DNA	242.25

Calculations:

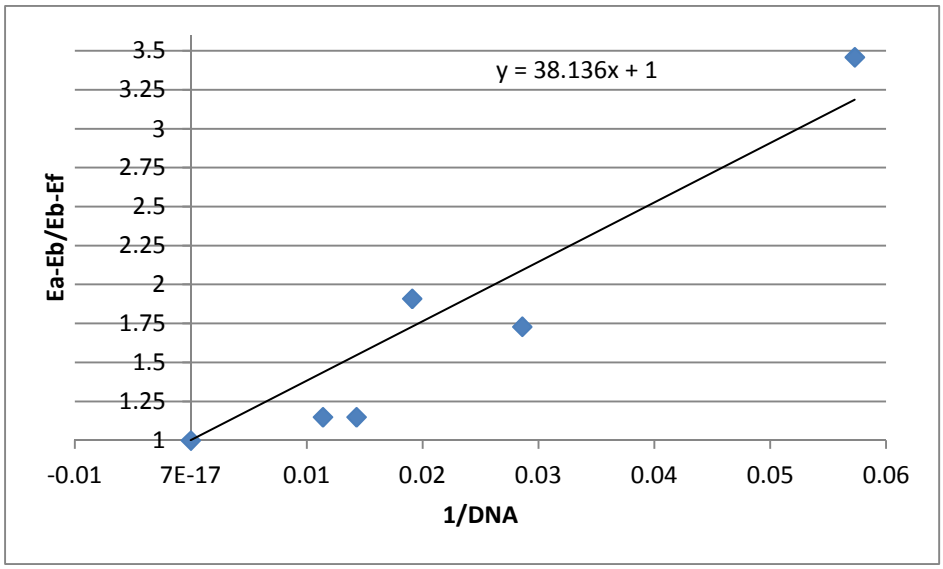
DNA Binding constat (K_b):

Table 4: Calculated values of 1/[DNA] and $(\varepsilon_a-\varepsilon_b)/(\varepsilon_b-\varepsilon_f)$

Vial	1/DNA	$(\varepsilon_a-\varepsilon_b)/(\varepsilon_b-\varepsilon_f)$
Vial 1	0.0573	3.46
Vial2	0.0286	1.73
Vial 3	0.0191	1.91
Vial 4	0.0143	1.15
Vial 5	0.0114	1.15
Vial 6	0.0095	0.68
Vial 7	0.0087	0.26

Vial 8	0.0071	0.15
Vial 9	0.0063	0.023
Vial 10	0.0057	0

Figure 1: Graph obtained from a plot of $(\varepsilon_a-\varepsilon_b)/(\varepsilon_b-\varepsilon_f)$vs 1/[DNA]



The plot of $(\varepsilon_a-\varepsilon_b)/(\varepsilon_b-\varepsilon_f)$ vs 1/[DNA] yields an intercept of one on y-axis and the reciprocal of the slope equals to binding constant value (K_b)

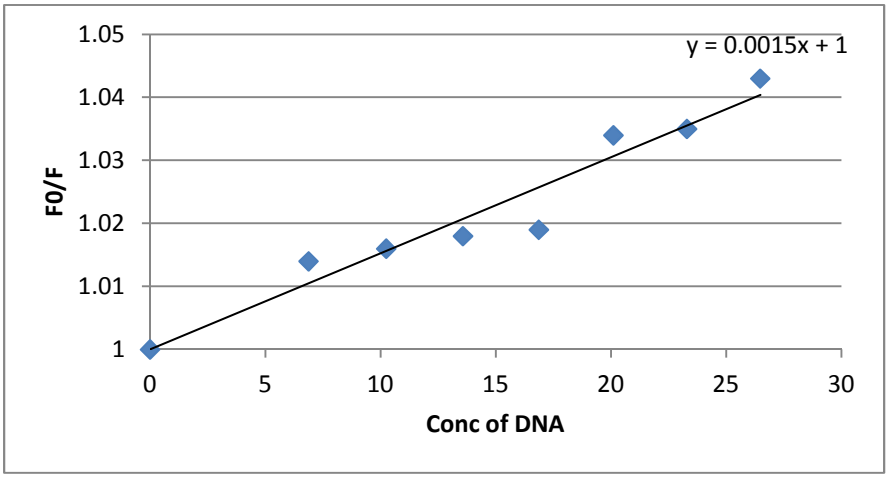
Stern Volmer Plot:

Table 5: Calculated values of F_0/F and concentration of DNA [Q].

S.no	Conc of DNA [Q]	F_0/F
1	3.46	1.012
2	6.88	1.014
3	10.25	1.016
4	13.58	1.018
5	16.87	1.019
6	20.11	1.034
7	23.3	1.035
8	26.48	1.043
9	0	1

The plot of F_0/F versus concentration of the quencher [Q] yields an intercept of one on y-axis and the slope of the line equals to quenching rate constant (Kq).

Figure 2: Graph obtained from the plot of F_0/F versus concentration of the quencher [Q]



Discussion:

Data was obtained from the UV spectroscopic and Fluorescence spectroscopic determinations. The Binding constant was determined using the slope of the line. Quenching experiments were conducted for each concentration at the specified quenching range, and the fluorescence of each solution containing quencher was measured. The maximum intensity for the solutions without quencher was seen at 530nm, and as a result, the intensities at this wavelength were measured. This procedure gave the ratio of Fo/F, which was plotted against the quencher concentration range. A fluorescence spectrum, Stern-Volmer plot were obtained the slope of the line determined the quenching rate constant value (Kq).

Conclusion:

The DNA binding constant value (K_b) for Humic acid was determined to be $2.6x10^4\ M^{-1}$ when compared to a known DNA binding molecule such as Ethidium Bromide (EtBr) which has a DNA binding constant value of $10^5\ M^{-1}$, the binding strength between HA and DNA is quite sturdy, However the fluorescent quenching rate constant (Kq) for Humic acid with CT-DNA as quencher was determined to be $1500\ M^{-1}$, which shows DNA is not an effective quencher. Therefore, according to our data the Humic acid strongly binds with DNA but the energy transfer between both the molecules are not significant. From the spectroscopic results of calf thymus DNA in the presence of HA in aqueous solution showed that there is a direct interaction between the HA and DNA, but the fluorescence resonance energy transfer (FRET) between the molecules was not very significant according to fluorometric data. The measurement of the interaction

between the potential drug and the target DNA is a subject of interest for drug development in future.

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