IN-VITRO STUDIES ON HYDROXAMIC ACID - CT DNA BINDING

Rubi Khilari and Rama Pande*

School of Studies in Chemistry, Pt.Ravishankar Shukla University, Raipur, (C.G.)-492010, India

E-mail: rubi.khilari796@gmail.com

Introduction

♦ As the basic genetic material of life, DNA is a particularly biological target for antiviral, anticancer and antibiotic drug.

♦ The study of interaction between DNA and a bioactive compound (drug) is important for understanding the mechanism of action and designing new DNA-targeted drug.

♦ Drugs bind to DNA both covalently and non-covalently. The possible mode of interactions between DNA and small molecules follows these models.

Major and Minor grooves in DNA molecule (B-Form)
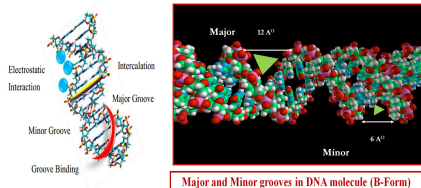
Hydroxamic acid molecules attract considerable attention because of their outstanding features namely.

1) Hydroxamic acids are drug like molecule follow "Lipinski Rule of Five".

2) Strong metal chelating ability.

3) -NO releasing properties.

4) Complexes with protein.

5) Active pharmacophoric part.

6) Antitumour/Anticancer agent.

7) Antioxidant property.

Experimental Section

HYDROXAMIC ACIDS	MOLECULAR WEIGHT	2D STRUCTURE	3D STRUCTURE
N-Phenyl 2,4dichloro phenoxy butyro	324.20		
N-m-tolyl 2,4 dichloro-phenoxyglutero	354.23		
N-m-tolyl-p-chloro phenoxyaceto	271.31		
N-m-chloro-phenyl-tertiarybutylbenzo	303.78		
N-p-tolyl-iso-valero	207.27		

WHY DNA IS TRAGETED ?

➤ DNA is a molecule of great biological significance as it is a common targets for antiviral, anticancer and antibiotic drugs.

➤ The total DNA content of a cell is termed as the 'Genome'. The 'Genome' is unique to an organism, and is the information bank governing all life processes of the organism, and stored as in form of DNA.

➤ DNA-binding drugs are designed to modulate gene activity and inhibit protein translation .

➤ In order to optimize the efficacy of drugs, as well as discover new drugs, it is important to fully characterize the drug-nucleic acid interaction, including sequence recognition and structural details of binding.

SOLUTION OF HYDROXAMIC ACIDS

The stock solutions of hydroxamic acids, (0.01M) were prepared in DMSO and used further of various concentrations as obtained by mass dilution technique. The final concentration of hydroxamic acids was prepared in Tris HCl.

PREPARTION OF CALF THYMUS

Calf thymus DNA was purchased from Genei and used without purification. The purity of DNA, free from protein was tested by absorption at A260/280. The DNA solution of ratio, less than 1.8 showed DNA free of protein. The final concentration through desired number of base pair was obtained at A260.

PREPARATION OF BUFFER

Doubly glass distilled water containing 50mM Tris-HCl and 50mM NaCl buffer at pH 7.8 was used. The pH of buffer was measured using a Cyber Scan 510 pH meter and adjusted by 0.01M HCl.

PREPARATION OF ETHIDIUM BROMIDE /LOADING BUFFER

Ethidium bromide was prepared in doubly glass distilled water containing 5µg/mL. Loading buffer contains 0.25% bromophenol + 0.25% xylene cyanol + 30% glycerol, in doubly glass distilled water.

PREPARATION OF GEL

0.8% of Agrose gel was prepared by dissolving appropriated amount of Gel in Tris EDTA Buffer.

Results and Discussion

UV METHOD

Absorption measurement is simple and effective in detecting a small molecule interacts with DNA. When an electron donor (D) interacts with an electron acceptor (A), a complex is formed. This interaction may be represented by the following equation,

$$[Ct\text{-}DNA] / (\varepsilon_a - \varepsilon_f) = [Ct\text{-}DNA] / (\varepsilon_b - \varepsilon_f) + 1/K_b (\varepsilon_b - \varepsilon_f)$$

where,

Ct- DNA = Base pair concentration,

ε_b, ε_f = Apparent absorption coefficients for bounded and free DNA,

K_b = Intrinsic binding constant,

The values of K_b of hydroxamic acids are obtained as ratio of slope $(1/(\varepsilon_a - \varepsilon_f)$ to intercept $(1/ K_b(\varepsilon_a - \varepsilon_f)$ by plotting graph between $[DNA] / (\varepsilon_a - \varepsilon_f)$ and $[DNA]$.
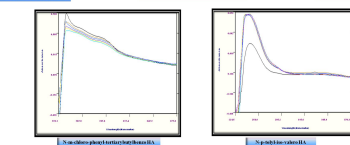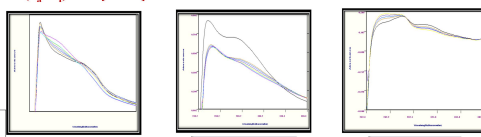
BINDING CONSTANT, K_b, OF HYDROXAMIC ACIDS - DNA COMPLEX

| HYDROXAMIC ACIDS	K_b M^{-1}	$Ksv M^{-1}$
N-phenyl 2,4dichloro phenoxy butyro	3.4×10^4	2.7×10^5
N-m-tolyl 2,4 dichlorophenoxy glutero	1.2×10^3	1.5×10^3
N-m-tolyl-p-chlorophenoxy aceto	1.5×10^7	9.9×10^4
N-m-chloro-phenyl-tertiarybutylbenzo	3.9×10^6	4.5×10^3
N-p-tolyl-iso-valero	3.6×10^5	1.6×10^5

VISCOMETERIC METHODS

In general, intercalation caused an increase in the viscosity of DNA solution due to lengthening of DNA helix as the base pair are pushed apart, and very little effect on the viscosity of DNA, if the electrostatic or groove surface binding occurs.

The viscosity of hydroxamic acids-DNA complexes are obtained by the expression,

$$\eta'_{sp} / \eta_{sp} = [(t_{complex} - t_0)/ t_0]/ [t_{control} - t_0 / t_0]$$

where,

η'_{sp} = the specific viscosity of DNA in the presence of the ligand.

η_{sp} = the specific viscosity of DNA in the absence of the ligand .

$t_{complex}$ = the average efflux times of complex.

$t_{control}$ = the average efflux times of DNA.

t_0 = the average efflux times of buffer.
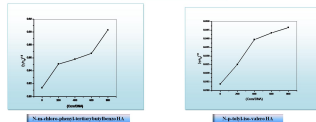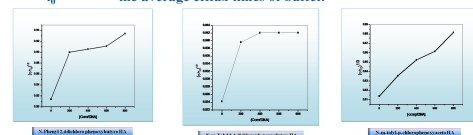
FLUOROMETRIC METHOD

❏ Fluorescence emission spectra of hydroxamic acid carried out in Cary Varian Spectrofluoremeter

❏ Fluorescence quenching is decrease in fluorescence intensity of luminescent species with interaction of other species.

❏ Binding constant K_{sv} were calculated by Stern-Volmer Equation .
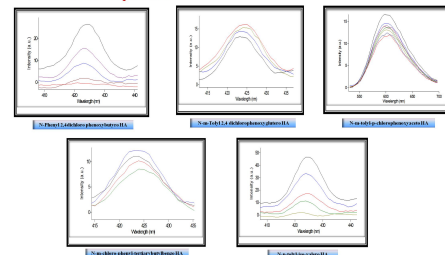
COMPETITIVE BINDING BETWEEN ETHIDIUM BROMIDE – DNA WITH HYDROXAMIC ACID

❏ Ethidium bromide is employed in the examination of the reaction, presumably binds initially to DNA through intercalation mode.

❏ This method is also used to elaborate binding mode.

❏ Like EtBr, if hydroxamic acids intercalate into the helix of DNA, it would compete with EtBr for the intercalation sites in DNA, and lead to a significant decrease in the fluorescence intensity of the DNA—EtBr complex.
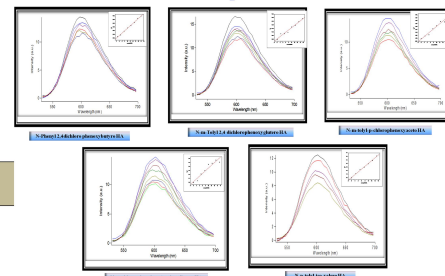
GEL ELECTROPHORESIS METHOD

When DNA (pBR322) is subjected to electrophoresis, a relatively fast migration will be observed for intact super coil, SC. If nicking occurs in one strand then the super coil will relax to generate a slower moving nicked coil, NC. If both the strands are cleaved a linear form that migrates between NC and SC is observed.
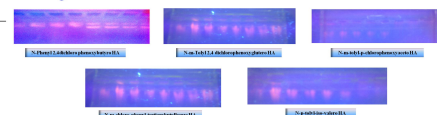
Conclusion

❏ The UV absorption intensity of hydroxamic acid-DNA complex has lower value than DNA.

❏ The relative viscosities of hydroxamic acid-DNA complexes have increased value as compared to DNA alone.

❏ The quenching of fluorescence for all the molecules is visible.

❏ The emission spectra decreases on adding hydroxamic acids.

❏ The DNA cleavage activity of some hydroxamic acid shows cleavage of pBR322 DNA.

REFERENCES

1) Lossen, H.; *Ann.Chem.*, 150, 314 (1869),

2) Agrawal, Y.K.; Tondon, S.G.; *J. Chem. Engg. Data*, 16, 495 (1971).

3) Pande, R.; Tondon, S.G.; *J. Chem. Engg. Data*, 24, 72 (1979).

4) Janjuaa, N. K.; Amber, S.; Yaquba, A.; Perveena, F.; Sabahata, S.; Mumtaza, M.; Jacobb, C.; Bab, L. A.; Hamdoon, A. M., *Spectrochimica Acta Part A* , 79, 1600-1604, (2011).

5) Raman, N.; Sobha, S.; Thamaraichelvan, A., *Spectrochimica Acta Part A: Molecular and Bio-*