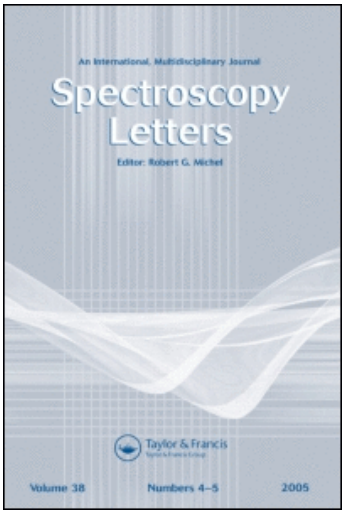

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A Study on the Interaction Between 6-Methyl Purine and Cytosine by Means of Absorption and Fluorescence Emission Spectroscopy

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A STUDY ON THE INTERACTION BETWEEN 6-METHYL PURINE AND CYTOSINE BY MEANS OF ABSORPTION AND FLUORESCENCE EMISSION SPECTROSCOPY

Key word : Hydrogen bond formation, 6-methyl purine

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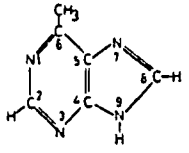
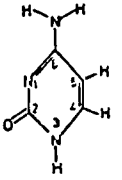
ABSTRACT

The hydrogen bond formation between 6-methyl purine and cytosine has been studied at room temperature. The absorption and fluorescence emission spectra of 2.10^{-5}M 6-methyl purine solutions containing different amounts of cytosine in the range of 2.10^{-4}-$3,5.10^{-4}$M were investigated. The association constant for hyrogen bonded complex formation between 6-methyl purine and cytosine was found to be $1,1.10^{3}$ M^{-1} from absorption difference spectra. The same association constant in the excited state was found to be $3,9.10^{3}$ M^{-1} from fluorescence spectra.

Intermolecular hydrogen bond formation can be studied by means of absorption and fluorescence emission methods. Ground state equilibrium constants of hyrogen bond formation can be found from absorption spectra[1-3]. A new and different equilibrium can be reached during the lifetime of the excited state, if the establishment of excited state equilibrium is faster enough than fluorescence emission[5]. In this case, excited state equilibrium constants can be calculated from fluorescence spectra[1,4,5]. In this study, the hydrogen bond formation between 6-methyl purine and cytosine has been investigated.



6-methyl purine



Cytosine

The hydrogen bond formation between 6-methyl purine (6-MPu) and cytosine (S) in ground state is written as follows:

$$6\text{-MPu} + S \rightleftharpoons 6\text{-MPu....S}$$

The association constant of this equilibrium can be given as follows

$$K = \frac{[6\text{-MPu...S}]}{[6\text{-MPu}][S]} \qquad (2)$$

K can be determined from equation (3) with the aid of absorption spectra.

$$\frac{1}{\Delta A} = \frac{1}{\Delta A_{max}} + \frac{1}{K \Delta A_{max}} \frac{1}{C_s} \qquad (3)$$

Where, ΔA: The difference in optical densities, K: The equilibrium constant of the hydrogen bonded complex formation in ground state, C_s: The cytosine concentration, ΔA_{max}: The maximum difference in optical densities.

On the other hand, the association reaction and the equilibrium constant in excited state can be given as:

$$(6\text{-MPu})^* + S \rightleftharpoons (6\text{-MPu....S})^* \qquad (4)$$

$$K^* = \frac{[6\text{-MPu....S}]}{[(6\text{-MPu})^*][S]} \qquad (5)$$

By using fluorescence emission method the K^* value can be estimated from the following equation.

$$\frac{(I/I_0)-1}{[S]} = \alpha K^* - K^*(I/I_0) \qquad (6)$$

Where, I, I_0: The fluorescence intensities of 6-methyl purine in presence and absence of cytosine respectively, [S]:Cytosine concentration, α : a constant, K^* : The equilibrium constant of the hydrogen bonded complex formation in excited state.

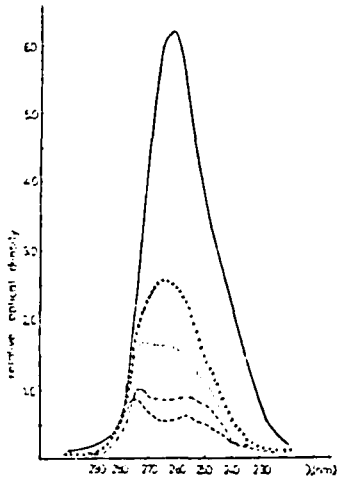


FIG.1. The absorption spectra of the 2.10^{-6} M 6-MPu solutions which contained different amounts of cytosine.
(—)without cytosine, (+++)2.10^{-4}M cytosine, (...)$2,5.10^{-4}$M cytosine, (-.-)3.10^{-4}M cytosine, (---)$3,5.10^{-4}$M cytosine.

EXPERIMENTAL

Chemicals: 6-methyl purine and cytosine were from Fluka(purum), bidistilled water was used as solvent.

Instrumentation: Absorption spectra were obtained using a Varian DMS 90 UV-Visible spectrometer, fluorescence spectra were measured by a Pica 55 spectrofluorometer.

RESULTS

1- The investigation of the hydrogen bond formation by means of absorption spectra:

The absorption spectra of the 2.10^{-5} M 6-methyl purine solutions, which contained different amounts of cytosine between 2.10^{-4} M - $3,5.10^{-4}$ M were obtained (FIG.1).

The differences in optical densities (ΔA) at different wavelengths and different cytosine concentrations were calculated and plotted against wavelength(FIG.2).

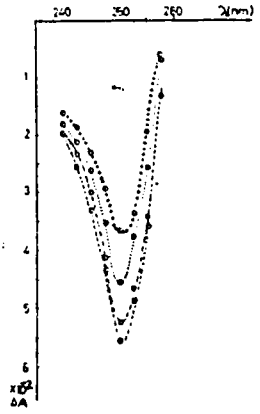


FIG 2. The absorption differences spectra of the 2.10^{-6}M 6-MPu solutions which contained different amounts of cytosine. (+++)2.10^{-4}M cytosine,(...)$2,5.10^{-4}$M cytosine, (-.-)3.10^{-4}M cytosine, (---)$3,5.10^{-4}$M cytosine.

TABLE 1

C_s(M)	ΔA	$1/C_s$	$1/\Delta A$
2.10^{-4}	-3,69	$50,00.10^2$	-0,271
$2,5.10^{-4}$	-4,56	$40,00.10^2$	-0,219
3.10^{-4}	-5,26	$33,33.10^2$	-0,190
$3,5.10^{-4}$	-5,57	$28,57.10^2$	-0,179

The change of ΔA values with cytosine concentration (C_s) at 260 nm are given in TABLE 1.

The graph of equation (3) , plotted by the method of least squares is shown FIG.3.

The equilibrium constant of the hydrogen bond formation in ground state was calculated to be $K=1,1.10^3$ M^{-1} from FIG.3.

2- The investigation of the hydrogen bond formation by means of fluorescence emission method:

Fluorescence emission spectra of 6-methyl purine solutions of which absorption spectra had been measured before,were obtained.

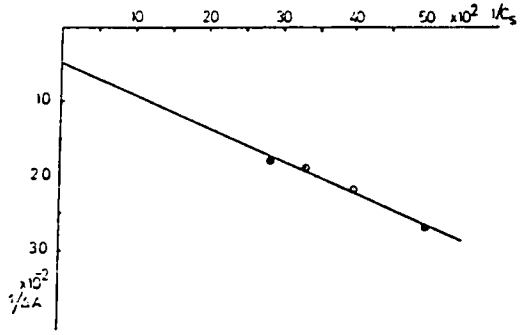
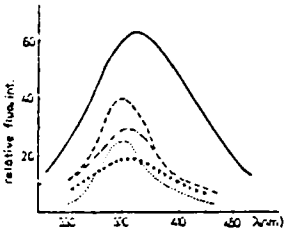


FIG.3.



(—)without cytosine
(---)10^{-4}M cytosine
(-.-)2.10^{-4}M cytosine
(...)$2,5.10^{-4}$M cytosine
(+++)$3,5.10^{-4}$M cytosine

FIG.4. The fluorescence spectra of the 2.10^{-5}M 6-MPu solutions which contained different amounts of cytosine.

TABLE 2

C_S(M)	-	10^{-4}	2.10^{-4}	$2,5.10^{-4}$	$3,5.10^{-4}$
I: relative fluorescence intensity at 370 nm	60	40	29	25	19
$(I/I_0)-1/C_S$	-	$-0,334.10^4$	$-0,258.10^4$	$-0,233.10^4$	$-0,195.10^4$

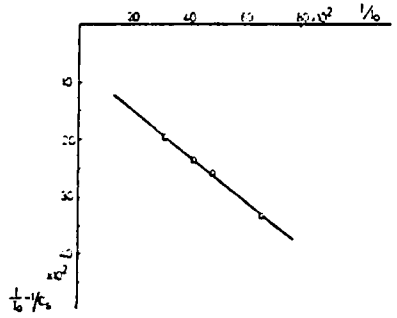


FIG.5.

Relative fluorescence intensities of each solution at 370nm are given in TABLE 2.

From the data in TABLE 2, $(I/I_0)-1/C_B$ versus I/I_0 plot was obtained (FIG.5).

From the graph in FIG.5, the equilibrium constant of the hydrogen bond formation in excited state was calculated to be $K^*=3,9.10^3$ M^{-1} according to the equation(6).

DISCUSSION

The hydrogen bond formation constant in the ground and excited states have been found to be $K=1,1.10^3 M^{-1}$ and $K^*=3,9.10^3$ M^{-1}. It appears that hydrogen bond between 6-methyl purine and cytosine becames stronger when 6-methyl purine molecule is excited. This result is in agreement with the finding[7] that 6-methyl purine is more acidic with respect to proton dissociation and more basic with respect to proton binding in excited state.

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