Binding of Tryptamine and 5-Hydroxytryptamine (Serotonin) to Nucleic Acids. Fluorescence and Proton Magnetic Resonance Studies*

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ABSTRACT: The fluorescence of tryptamine, 5-hydroxytryptamine (serotonin), and 5-methoxytryptamine is quenched upon binding to nucleic acids. The fluorescence lifetime is not modified and binding curves show that bound tryptamine or serotonin molecules do not fluoresce. Fluorescence studies of mixed aggregates of tryptamine (or serotonin) and nucleosides in frozen aqueous solutions demonstrate that fluorescence is quenched as a result of charge transfer from the indole ring to the purine or pyrimidine rings. In aqueous solutions, absorption and proton magnetic resonance studies demonstrate that indole derivatives and nucleosides form intermolecular complexes in which the two aromatic rings are stacked. Proton magnetic resonance studies of the binding of serotonin to poly (A) show that the indole and adenine rings are stacked in the complex. Besides electrostatic interactions between charged groups, the binding of tryptamine and serotonin to nucleic acids involves overlapping of the electron clouds of the indole ring and the bases. This stacking interaction occurs not only with native DNA but also with denatured DNA and single-stranded polynucleotides. These results are discussed with respect to the role that could be played by tryptophan residues of proteins and enzymes in the binding to nucleic acids and to the possible involvement of nucleic acids in the serotonin receptor site in the brain.

The binding of enzymes or proteins to nucleic acids involves not only electrostatic forces between charged groups but also more specific interactions between amino acid residues and bases (Yarus, 1969, and references cited therein). Interactions of the aromatic rings of aromatic amino acids with the purine or pyrimidine rings could ensure a specific positioning of the nucleic acid with respect to the protein molecule (Hélène, 1971). In previous publications, we showed that tryptophan could interact with nucleic acid bases either in mixed aggregates obtained in frozen aqueous solutions (Montenay-Garestier and Hélène, 1968, 1971) or in concentrated aqueous solutions (Dimicoli and Hélène, 1971). We report here the results of a fluorescence study of the interactions between tryptamine derivatives and nucleic acids. Intercalation of the indole ring between the bases leads to stacked complexes in which indole fluorescence is quenched. Substitution of the indole ring by an hydroxyl group in position 5 leads to a red shift of its absorption spectrum. This facilitates fluorescence studies since this substituted molecule can be excited at wavelengths where DNA does not absorb. Moreover, among indole derivatives of biological interest, 5-hydroxytryptamine (serotonin) plays an important role as a synaptic transmitter (Bradley, 1968) and as a growth stimulator (Boucek and Alvarez, 1969). There is also evidence that hallucinogenic drugs whose structure is related to tryptamine act primarily on the central serotonin mechanism (Smythies, 1970). In view of the recently reported observation that serotonin could bind to nucleic acids (Smythies and Antun, 1969), we have studied in some details the mechanism of serotonin interaction with native and denatured DNAs and also with the components of nucleic acids. The results obtained with tryptamine and serotonin are shown to be quite similar.

Experimental Section

Tryptamine hydrochloride, serotonin oxalate, and nucleosides were purchased from California Corp. for Biochemical Research.

5-Methoxytryptamine was obtained from Aldrich.

Calf thymus DNA and poly[d(A-T)] were kindly supplied by Dr. G. Aubel-Sadron. *Micrococcus lysodeikticus* DNA was a gift from Dr. M. Leng. Polyadenylic acid was purchased from Schwarz Biochemicals.

Solutions were made in the following buffer: sodium cacodylate (1 mM)–sodium chloride (1 mM), pH 7. Ionic strength was changed by adding sodium chloride.

Fluorescence measurements were carried out with a Jobin-Yvon spectrophotometer. The sample was contained in a quartz cell thermostated at 15 ± 1°. To take into account fluctuations in lamp intensity, the fluorescence of the sample was compared to that of a reference solution containing tryptamine or serotonin. Each measurement was repeated at least three times. For low-temperature measurements (77°K), the sample was frozen in a 2-mm i.d. quartz tube. Fluorescence lifetimes were measured using the single-photon counting technique (Wahl, 1969).

Absorption spectra were recorded with a Cary 14 spectrophotometer.

Proton magnetic resonance spectra were recorded with a Brücker HFX 90-MHz spectrometer equipped with a Fabri Tek 1072 computer. All chemical shifts were measured with respect to an external reference and corrections for changes in bulk magnetic susceptibility were made when necessary (Dimicoli and Hélène, 1971).

Results

Fluorescence Quenching upon Binding of Tryptamine and Serotonin to Native DNA. The fluorescence maxima of tryptamine and serotonin in aqueous solutions at pH 7 are 360 and 342 nm, respectively. Under our experimental conditions

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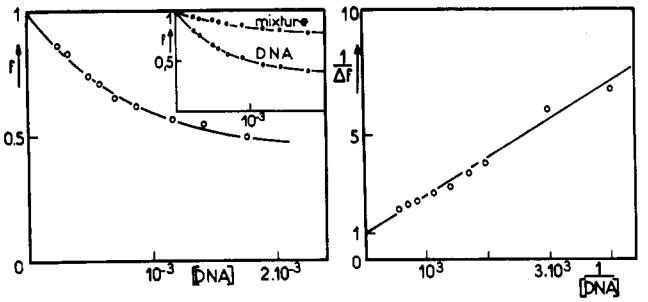

FIGURE 1: Quenching of serotonin fluorescence by calf thymus DNA (excitation = 305 nm, emission = 340 nm, serotonin concentration 10^{-5} M). Left part: the relative fluorescence quantum yield, f, of serotonin is plotted against DNA concentration (expressed in moles of phosphate per liter). Insert: variation of the *apparent* fluorescence quantum yield of serotonin in the presence of DNA and a mixture of nucleosides having the same absorbance at the excitation wavelength (305 nm). The true relative quantum yield was taken as the ratio of the values obtained from these two curves (to take into account the inner filter effect of DNA). Right part: plot of data from the left part according to eq 6.

(concentrations less than 2×10^{-5} M), the longest wavelength at which tryptamine and serotonin molecules can be excited are about 295 and 305 nm, respectively. Addition of calf thymus DNA leads to a decrease in the fluorescence intensity. Since rather high DNA concentrations are used, the screening effect of DNA at the excitation wavelength has to be taken into account, especially in the case of tryptamine. (Smythies and Antun (1969) who already observed fluorescence quenching did not correct their data for this screening effect.) The fluorescence intensity of tryptamine or serotonin in the presence of DNA was compared to that in the presence of a mixture of nucleosides corresponding to the base composition of DNA and having the same absorbance at the excitation wavelength. Independent experiments showed that interaction of tryptamine or serotonin with nucleosides is too weak to give a measurable quenching of fluorescence in the concentration range investigated here (see below and Dimicoli and Hélène 1971). Once the corrections are made, the change in fluorescence quantum yield with DNA concentration can be determined (Figure 1). These measurements show that the fluorescence quantum yield of tryptamine or serotonin is decreased as a result of binding to DNA.

Assuming several binding processes, the overall quantum yield ϕ in the presence of DNA is given by

$$\phi = \phi_f \frac{C_f}{C_t} + \sum_i \phi_b{}^i \frac{C_b{}^i}{C_t} \qquad (1)$$

where ϕ_f is the fluorescence quantum yield of free serotonin molecules (concentration C_f) ,$\phi_b{}^i$ is the fluorescence quantum yield of serotonin molecules bound to sites of type i (concentration $C_b{}^i$).

$$C_t = C_f + \sum_i C_b{}^i$$

is the total serotonin concentration.

Fluorescence lifetimes were measured in the absence and the presence of DNA. At a concentration of DNA that gave 50% quenching, no change in fluorescence lifetime was observed. This is shown on Figure 2 in the case of serotonin whose fluorescence lifetime remains 4.1 nsec in the presence of 2×10^{-3} M DNA.

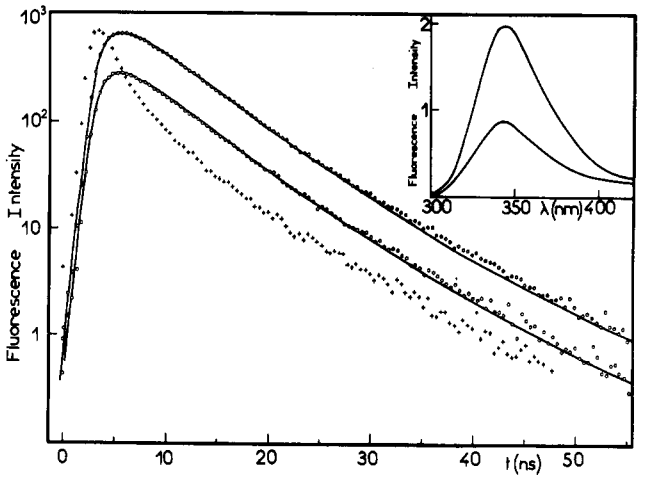

FIGURE 2: Fluorescence decay under flash excitation of serotonin 2×10^{-5} M in the absence (upper curve) and the presence (lower curve) of 2×10^{-3} M DNA (the corresponding fluorescence spectra are shown inset). Crosses represent the flash profile. The full lines are calculated for a single exponential decay with a lifetime of 4.1 ns. The circles are the experimental points.

Hence, $\phi_b{}^i$ must be equal either to ϕ_f or to 0. Thus assuming two classes of binding sites ($\phi_b{}^1 = \phi_f$, $\phi_b{}^2 = 0$), eq 1 can be written as

$$\phi = \phi_f \frac{C_f + C_b{}^1}{C_t} \qquad (2)$$

If the two classes of binding processes are independent of each other and if the binding sites are independent and equivalent within each class, the concentrations of bound molecules are given by

$$C_b{}^1 = \frac{n_1 K_1 P C_f}{1 + K_1 C_f} \qquad (3)$$

$$C_b{}^2 = \frac{n_2 K_2 P C_f}{1 + K_2 C_f} \qquad (4)$$

where P is DNA concentration expressed in moles per liter of phosphate, n_i is the number of binding sites in class i, and K_i the average association constant for that class.

Under most of our experimental conditions, the total amine concentration (C_t) remains low as compared to phosphate concentration. One can assume that $r_i = C_b{}^i/P$ is small as compared to n_i (the number of occupied sites is small as compared to the number of available sites). Equation 2 can be written as

$$\phi = \phi_f \frac{1 + K_1 n_1 P}{1 + (K_1 n_1 + K_2 n_2) P} \qquad (5)$$

The above assumption ($r_i \ll n_i$) is experimentally supported by the observation that at a given phosphate concentration and at low amine concentrations, the fluorescence quantum yield is independent of amine concentration (eq 5). In Figure 5, it can be seen that the fluorescence intensity increases linearly with serotonin concentration in the absence as well as in the presence of DNA. Only the slope is reduced in the presence of DNA.

TABLE I: Values of the Product $K \times n$ (M^{-1})[a] for Serotonin Binding to Nucleic Acids at 15° in Cacodylate Buffer (1 mM) Sodium Chloride (1 mM) at pH 7.

ML DNA[b]	CT DNA	Poly-[d(A-T)]	Denatured CT DNA	Poly(A)
660	710	805	310	460

[a] The value of Kn for tryptamine binding to CT DNA is 260 M^{-1}. [b] ML *Micrococcus lysodeikticus;* CT calf thymus.

Equation 5 can be modified to give

$$\frac{1}{\Delta f} = \frac{\phi_f}{\phi_f - \phi} = \frac{K_1 n_1 + K_2 n_2}{K_2 n_2} + \frac{1}{K_2 n_2} \frac{1}{P} \qquad (6)$$

where f represents the relative quantum yield ϕ/ϕ_f and Δf is equal to $1 - (\phi/\phi_f)$. Thus a plot of $1/\Delta f$ against $1/P$ should give a straight line whose slope and y axis intercept could be used to obtain the values of $K_1 n_1$ and $K_2 n_2$. Such a plot for serotonin–DNA and tryptamine–DNA mixtures are shown in Figures 1 and 3, respectively. Extrapolation gives a y-axis intercept equal to 1 in both cases. This means that $K_1 n_1$ is small as compared to $K_2 n_2$. Thus, in the range of concentrations investigated, every tryptamine or serotonin molecule bound to DNA belongs to a class 2 site which is not fluorescent. Values of $K_2 n_2$ are given in Table I. The values of n_2 can be determined using higher amine to phosphate ratios. Analysis of the fluorescence data was made according to Scatchard (1949), plotting r/C_f against r where $r = C_b/P$.

$$\frac{r}{C_f} = K(n - r) \qquad (7)$$

C_b and C_f were determined from the measurement of the overall fluorescence quantum yield. Although the measurements are not very accurate at high serotonin concentrations because of important inner filter effects, values of n were estimated to be around 0.15. Figure 3 shows such a Scatchard plot (eq 7) for serotonin binding to calf thymus DNA.

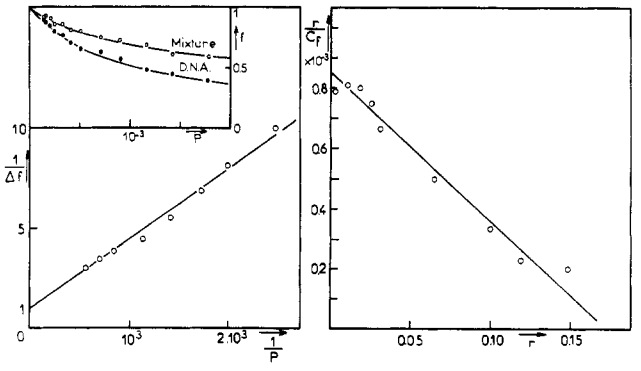

FIGURE 3: Left part: quenching of tryptamine fluorescence by calf thymus DNA. Inset is shown the apparent relative fluorescence quantum yield in the presence of DNA and of a mixture of nucleosides having the same absorbance at the excitation wavelength (295 nm). Fluorescence intensity is measured at 360 nm. The data are then plotted according to eq 6. Right part: Scatchard plot for the binding of serotonin to calf thymus DNA (see text).

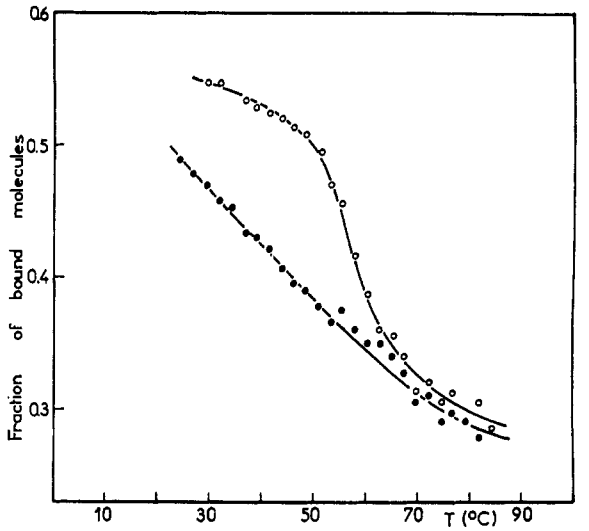

FIGURE 4: Temperature dependence of the fraction of serotonin molecules bound to poly(A) (●) and calf thymus DNA (○). The fraction of bound serotonin was determined from the extent of fluorescence quenching at each temperature (taking into account the change in fluorescence quantum yield of free serotonin with temperature).

Methylation of the hydroxyl group of serotonin does not prevent the binding to nucleic acids. The fluorescence of 5-methoxytryptamine is quenched to the same extent as that of 5-hydroxytryptamine, thus suggesting that the hydroxyl group of serotonin bound to DNA is not involved in hydrogen bonding.

To determine whether the binding of serotonin was dependent upon base composition, *Micrococcus lysodeikticus* DNA [28% (A-T)], calf thymus DNA [52% (A-T)], and crab poly-[d(A-T)] [97% (A-T)] were compared. The values of Kn increase slightly with the (A-T) content of DNA (Table I). Only the product of the association constant K and the number of binding sites n were measured, so that it is not possible to draw any definite conclusion about base specificity.

Temperature Dependence of Binding. The binding of serotonin to DNA is temperature dependent as shown in Figure 4. The fluorescence quantum yield of serotonin and tryptamine decreases when temperature increases. At each temperature, the amount of bound molecules was determined from the extent of fluorescence quenching. The percentage of bound molecules decreases slightly up to about 50° at which temperature DNA begins to undergo its transition from the double-stranded to the single-stranded form under our experimental conditions. During the melting process (T_m = 65° determined from absorbance measurements), more serotonin is released from the complex but the amount of serotonin bound to DNA at 85° is still important although more than 95% of the double-helical structure is destroyed. This suggested that denatured DNA could bind serotonin.

Binding to Single-Stranded Polymers. DNA was denatured by heating at 90° for 10 min and then quickly cooled down to 0°. The fluorescence of serotonin and tryptamine is quenched by denatured DNA and the value of the product $K \times n$ is only about twice smaller at 20° (Table I). Single-stranded polynucleotides such as poly(A) at pH 7 also bind serotonin and tryptamine (Figure 5).

The binding of tryptamine and serotonin to denatured DNA and to single-stranded poly(A) at pH 7 was studied as a func-

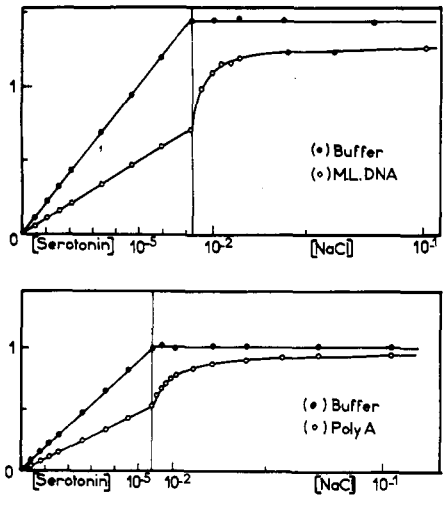

FIGURE 5: Binding of serotonin to *Micrococcus lysodeikticus* DNA 1.5×10^{-3} M (upper part) and to poly(A) 1.9×10^{-3} M (lower part). Fluorescence intensity increases linearly with serotonin concentration in the absence (●) and in the presence (○) of DNA or poly(A). The pH 7 buffer contains sodium cacodylate (1 mM)-sodium chloride (1 mM). The fluorescence quantum yield of free serotonin is not affected by added sodium chloride (●). Addition of sodium chloride to the mixture of serotonin and DNA or poly(A) releases serotonin from the complex (○).

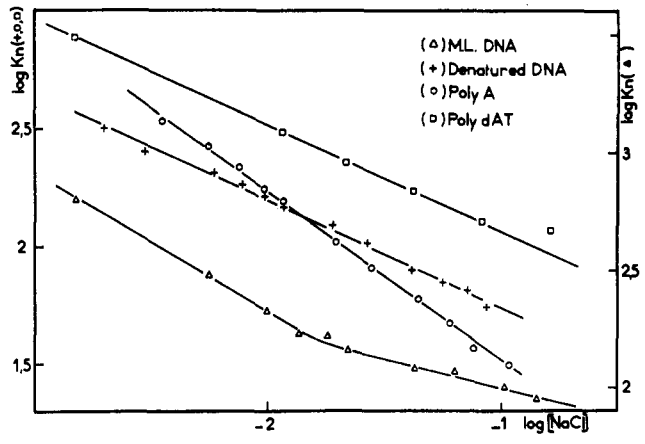

FIGURE 6: Ionic strength dependence of the product $K \times n$ (log − log plot) for denatured DNA (+), poly(A) (○), poly[d(A-T)] (□) (left scale) and for native *Micrococcus lysodeikticus* DNA (△) (right scale).

tion of temperature (Figure 4). The percentage of bound serotonin molecules determined from fluorescence quenching decreases smoothly with temperature and does not reveal any cooperative transition as this is observed with native DNA. The decrease in the amount of bound molecules when temperature increases could not only be due to a change in the association constant but also to a modification in the number of binding sites which could accompany the decrease in helical content of the polymer as the temperature is raised.

Ionic Strength Effects upon Binding. Since tryptamine and serotonin possess positively charged amino groups, the binding of these molecules to DNA should involve electrostatic interaction with the negatively charged phosphate groups. This electrostatic contribution to the binding process is demonstrated by the effect of ionic strength upon fluorescence quenching. Increasing sodium chloride concentration leads to an increase of the overall fluorescence quantum yields of serotonin in the presence but not in the absence of DNA (Figure 5). Thus serotonin is released from the complex as ionic strength increases.

Ionic strength effects on the binding of small organic cationic molecules to polyelectrolytes have been already described (Chambron *et al.*, 1966). The association constant, K, should change with ionic strength, I, according to the following relationship:

$$\log K = C + \alpha \log I \qquad (8)$$

where C and α are constants.

Since most of our fluorescence measurements yield only the values of the product $K \times n$, we have plotted in Figure 6 log Kn *vs.* log I. For denatured DNA, poly[d(A-T)] and poly(A), log Kn varies linearly with log I. This is not the case with native DNA. These differences might be ascribed to a change in the value of n with ionic strength in the case of native DNA but not in the case of single-stranded polymers and of the alternated poly[d(A-T)]. Ionic strength effects upon the number of binding sites have already been reported for the binding of cationic dyes to DNA (see, for example, Chambron *et al.*, 1966).

Electrostatic contribution to the stability of aromatic amine complexes with DNA is also demonstrated by the weak interaction of indole or 5-hydroxyindole as compared to tryptamine or 5-hydroxytryptamine.

Formation of Electron Donor–Acceptor Complexes between Indole Derivatives and Nucleic Acid Components. External binding of the N^+H_3 group of tryptamine or serotonin to the phosphate groups of DNA would not be expected to completely quench the fluorescence of the indole ring. In the concentration range investigated, phosphate ions themselves or mononucleotides have no quenching effect on serotonin fluorescence. One could envisage that fluorescence quenching is due to a stacking of the indole rings outside the double helix as observed in the type II complex of acridine dyes with DNA (Stone and Bradley, 1961). However stacking interactions do not appear to quench the fluorescence of indole derivatives since stacked aggregates obtained in frozen aqueous solutions are still highly fluorescent (see below, Figure 7).

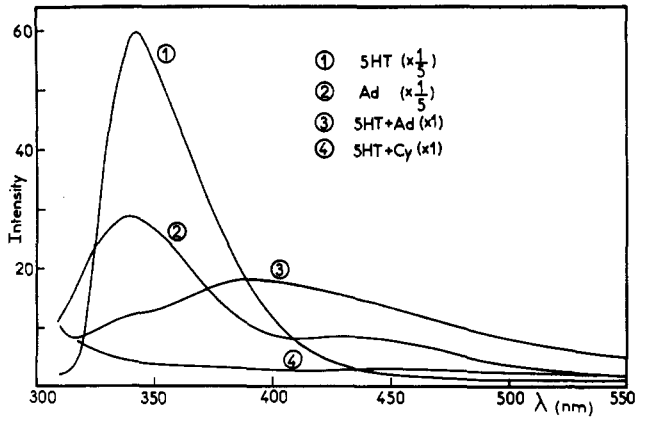

FIGURE 7: Total luminescence spectra of frozen aqueous solutions (10^{-3} M) at 77°K of 5-hydroxytryptamine (5HT), adenosine (Ad), and the equimolar mixtures of 5-hydroxytryptamine and adenosine or cytidine. Intensities are multiplied by the factors indicated on the figure.

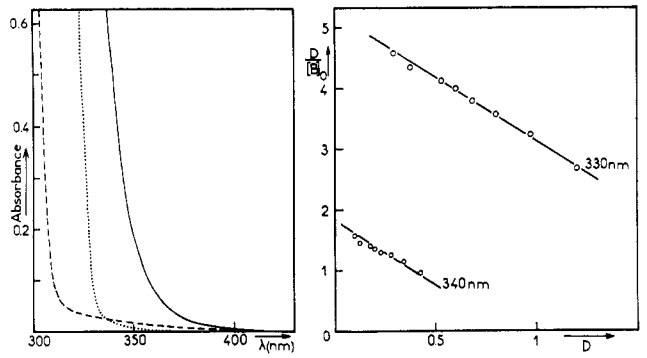

FIGURE 8: Complex formation between uridine and 5-hydroxytryptamine (serotonin) in cacodylate buffer (1 M) at pH 7. Left: absorption spectra in 1-cm cells of uridine (0.45 M) (- - -), serotonin (0.01 M) (. . .), and the corresponding mixture (—). Right: analysis of absorbance data according to eq 9. $[B]_0$ is the total concentration of uridine and D the absorbance at the indicated wavelength. The total concentration of serotonin ($[A]_0$) remains constant and equal to 0.01 M.

TABLE II: Association Constants for Complex Formation between 5-Hydroxytryptamine (5HT) or Tryptamine (T) and Nucleosides or Nucleotides in Aqueous Solutions at 22°.

Complex	Method[a]	K (M^{-1})	Conditions[b]
5HT + cytidine	Abs	1.7	pH = 7, I = 1
5HT + uridine	Abs	2.1	pH = 7, I = 1
T + UMP	Pmr	1.8	pD = 7.9, I = 0.2
T + AMP	Pmr	6.4	pD = 4.9, I = 0.1
T + adenosine	Pmr	3.3	pD = 5, I = 0.1

[a] Association constants were determined from absorbance (abs) or proton magnetic resonance (pmr) measurements (see Dimicoli and Hélène, 1971). [b] Absorbance data were obtained in sodium cacodylate buffer (1 M) at pH 7. Pmr experiments were performed in D_2O at the pD and ionic strength (I) values indicated.

We previously reported that the fluorescence of tryptamine is quenched in mixed aggregates with nucleosides (see Figure 9 in Montenay-Garestier and Hélène, 1971). Such mixed aggregates can be obtained when an equimolar aqueous mixture of serotonin and a nucleoside is frozen down. Stacking interactions in mixed aggregates were previously demonstrated from triplet–triplet energy-transfer measurements (Hélène and Montenay-Garestier, 1968). In Figure 7 it can be seen that serotonin fluorescence is quenched in mixed aggregates with nucleosides. A weak fluorescence at longer wavelengths is observed with purine derivatives whereas no fluorescence at all is obtained with pyrimidine nucleosides (Figure 7). The results are thus quite similar to those obtained with tryptamine and already ascribed to electron transfer from the indole ring to the purine or pyrimidine ring of the nucleoside. Pyrimidines behave as better electron acceptors than purines (Montenay-Garestier and Hélène, 1968, 1971). The quenching of serotonin fluorescence by nucleosides is more efficient than that of tryptamine as would be expected since the presence of a hydroxyl group in position 5 in the indole ring should enhance its electron donor ability.

The formation of electron donor–acceptor complexes between indole derivatives and nucleic acid bases can also be demonstrated in concentrated aqueous solutions by absorption spectroscopy and by proton magnetic resonance (pmr) (Dimicoli and Hélène, 1971). The mixtures of nucleosides and indole derivatives in aqueous solutions at pH 7 absorb at longer wavelengths than the separated components (Figure 8). The effect is much more important with pyrimidine than with purine nucleosides. The method of continuous variations (Job, 1928) allows us to determine the stoichiometry of the complexes. The corresponding plots (Job plots) indicate that these complexes have essentially a 1:1 stoichiometry. Absorbance data can then be analyzed according to the following relationship derived when one of the components (B) is in large excess as compared to the other (A) (Foster *et al.*, 1953).

$$\frac{D^{\lambda}}{[A]_0[B]_0} = \frac{1}{\epsilon_{AB}{}^{\lambda} \times l} + \frac{1}{\epsilon_{AB}{}^{\lambda} \times l} \frac{1}{K} \frac{D}{[A]_0} \qquad (9)$$

where D^{λ} is the absorbance of the mixture at a wavelength λ where only the complex AB absorbs, $\epsilon_{AB}{}^{\lambda}$ is the extinction coefficient of the complex AB at the same wavelength λ, and l the optical path of the cell used for absorbance measurements. K is the equilibrium constant for complex formation $A + B \rightleftarrows AB$. $[A]_0$ and $[B]_0$ are the total concentrations of compounds A and B ($[B]_0 \gg [A]_0$). Analysis of the absorbance data according to eq 9 permits the determination of K (see Table II and Figure 8).

Complex formation between tryptamine, serotonin, or other indole derivatives and nucleosides or nucleotides leads to upfield shifts of the proton magnetic resonance (pmr) signals of both aromatic rings (Dimicoli and Hélène, 1961). For example, it can be seen on Figure 9 that the H_8 and H_2 resonances of AMP are shifted toward higher fields in the presence of tryptamine. These upfield shifts are due to ring current effects of one ring on to the other and imply that the two aromatic rings are stacked in the complex (Foster and Fyfe, 1965). The ribose protons are also displaced upfield in the presence of indole derivatives. The largest displacement is observed for $H_{1'}$ which is the closest to the purine or pyrimidine ring. Complex formation also affects the ribose conformation as shown by a decrease in the coupling constant $J_{H_{1'}H_{2'}}$ between $H_{1'}$ and $H_{2'}$ protons. For example, this coupling constant for adenosine (10^{-2} M) is 6.2 Hz in the absence and 4.8 Hz in the presence of 10^{-1} M tryptamine. These results are discussed in more details in another publication (Dimicoli and Hélène, 1971).

If the exchange rate between free and complexed molecules is fast enough (on the nmr time scale), pmr data can be analyzed according to the following relationship derived when one of the components (B) is in large excess as compared to the other (A) (Foster and Fyfe, 1965).

$$\frac{\Delta\delta}{[B]_0} = -K(\Delta\delta - \Delta\delta_{AB}) \qquad (10)$$

where $\Delta\delta = \delta - \delta_A$ and $\Delta\delta_{AB} = \delta_{AB} - \delta_A$, δ_A and δ_{AB} are the chemical shifts of a proton of molecule A in the free and the complexed state, respectively. δ is the measured chemical shift of the same proton in the presence of compound B at a concentration $[B]_0$. Plots of pmr data according to eq 10 gives straight lines (Figure 9) whose slopes yield association constants (Table II) (see also, Dimicoli and Hélène, 1971).

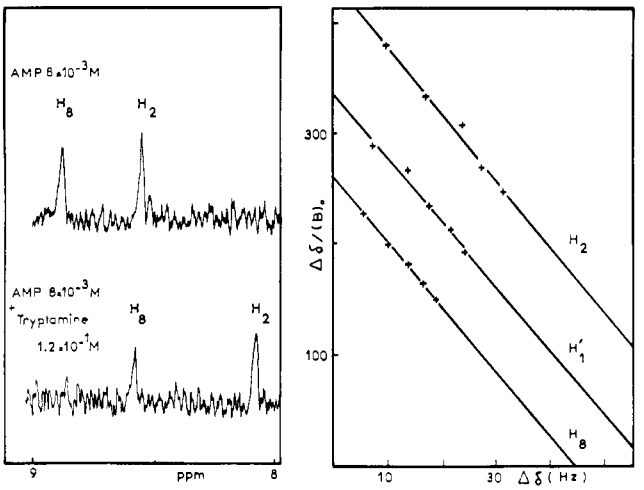

FIGURE 9: Left part: resonance signals of the H_8 and H_2 protons of AMP in the absence and the presence of 0.12 M tryptamine. Right part: analysis of pmr data according to eq 10 for complex formation between AMP and tryptamine at pD 6 at constant ionic strength (0.1).

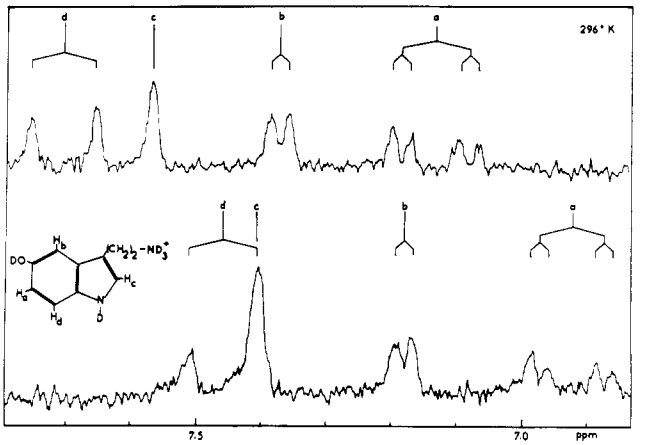

FIGURE 10: Pmr spectra (aromatic protons) of serotonin 10^{-2} M at pD 7 in the absence (upper spectrum) and the presence (lower spectrum) of poly(A) (10^{-2} M) (expressed in moles of phosphate per liter). The pmr spectrum of the aromatic protons of substituted 5-hydroxyindole derivatives has been analyzed by Biaglow *et al.* (1969).

The new absorption observed at long wavelengths upon mixing an indole derivative with a nucleoside has been ascribed to electron transfer from the indole ring to the purine or pyrimidine ring in the stacked complex (Dimicoli and Hélène, 1971). Charge-transfer contribution is enhanced in the excited state as observed in most weak electron donor–acceptor complexes (Mataga and Murata, 1969) and this leads to fluorescence quenching (Montenay-Garestier and Hélène, 1971).

Proton Magnetic Resonance Studies of Poly(A)–Serotonin Interactions. The main features of the pmr spectrum of single-stranded poly(A) at pH 7 have been already discussed (McDonald *et al.*, 1964; Jardetzky, 1964; McTague *et al.*, 1964). The H_2 and H_8 resonances of the adenine ring are displaced upfield and broadened as compared to the monomer AMP. Increasing the temperature leads to downfield shifts and the resonance peaks become narrower (McDonald *et al.*, 1964) (see Figure 11). When serotonin is added to a poly(A) solution at pH 7, the resonances of the serotonin protons undergo upfield shifts (Figure 10). The magnitude of these shifts depends on the concentration of poly(A), the temperature of the sample and the particular proton investigated. These upfield shifts are similar to those observed in the stacked complexes formed between indole derivatives and purine nucleosides which have been ascribed to ring-current effects (see earlier and Dimicoli and Hélène, 1971). At room temperature (296°K) the H_8 resonance of the adenine ring in poly(A) undergoes a downfield shift in the presence of serotonin whereas the H_2 resonance peak is displaced upfield (Figure 11). At higher temperatures (310°K and above), both the H_8 and the H_2 resonances are shifted upfield. This behavior of the H_8 and H_2 resonances in poly(A) upon binding of serotonin can be considered as resulting from two opposite effects: (i) ring current effects of the indole ring in stacked complexes will shift the resonances of the adenine ring protons toward higher fields; (ii) a decrease in adenine–adenine interactions due to intercalation of serotonin between adenine bases will lead to downfield shifts of the adenine proton resonances. The net result of these two opposite effects will depend on the temperature since adenine–adenine interactions decrease markedly with temperature (McDonald *et al.*, 1964) while the amount of bound serotonin decreases slowly when the temperature increases (see earlier). Thus pmr studies of serotonin binding to poly(A) clearly demonstrate that serotonin interacts with adenine bases to form stacked complexes at the expense of adenine–adenine interactions. This is further supported by the narrowing of the adenine resonance peaks upon binding of serotonin (Figure 11). The interaction of tryptamine with poly(A) induces changes in the pmr spectra of both compounds quite similar to those observed with serotonin.

Discussion

The fluorescence of tryptamine and serotonin is quenched when these molecules interact with DNA in its native or denatured state. Electrostatic interaction between the positively charged amino groups of these aromatic amines and the negatively charged phosphate groups of DNA is involved in the binding process as shown by the dependence of binding constant upon ionic strength.[1] However, such an interaction is not expected to affect the fluorescence of indole ring. The study of monomer mixtures demonstrates that an interaction between the aromatic rings of indole derivatives and nucleic acid components takes place in aqueous solution. This stacking interaction leads to a possibility of electron transfer from the indole ring to the base. Although it is likely that charge transfer does not contribute greatly to the stabilization of the complexes in their ground state (Malrieu and Claverie, 1968), its contribution is enhanced in the excited state. This leads to a quenching of indole fluorescence as observed in mixed aggregates.

Thus fluorescence quenching of tryptamine and serotonin bound to DNA is very likely due to an interaction between the

[1] Changes in ionic strength also affect phosphate–phosphate interactions. This can lead to a decrease in the association constant for the binding of uncharged molecules, such as hydrocarbons, to DNA (Lesko *et al.*, 1968). However, most of the effects observed here take place at low ionic strength (<0.05 M), and removal of the positive charge (as in 5-hydroxyindole compared to serotonin) practically suppress fluorescence quenching in the presence of DNA. Ionic strength effects can thus be considered as resulting mainly from a competition between metal ions and amines for the DNA phosphates.

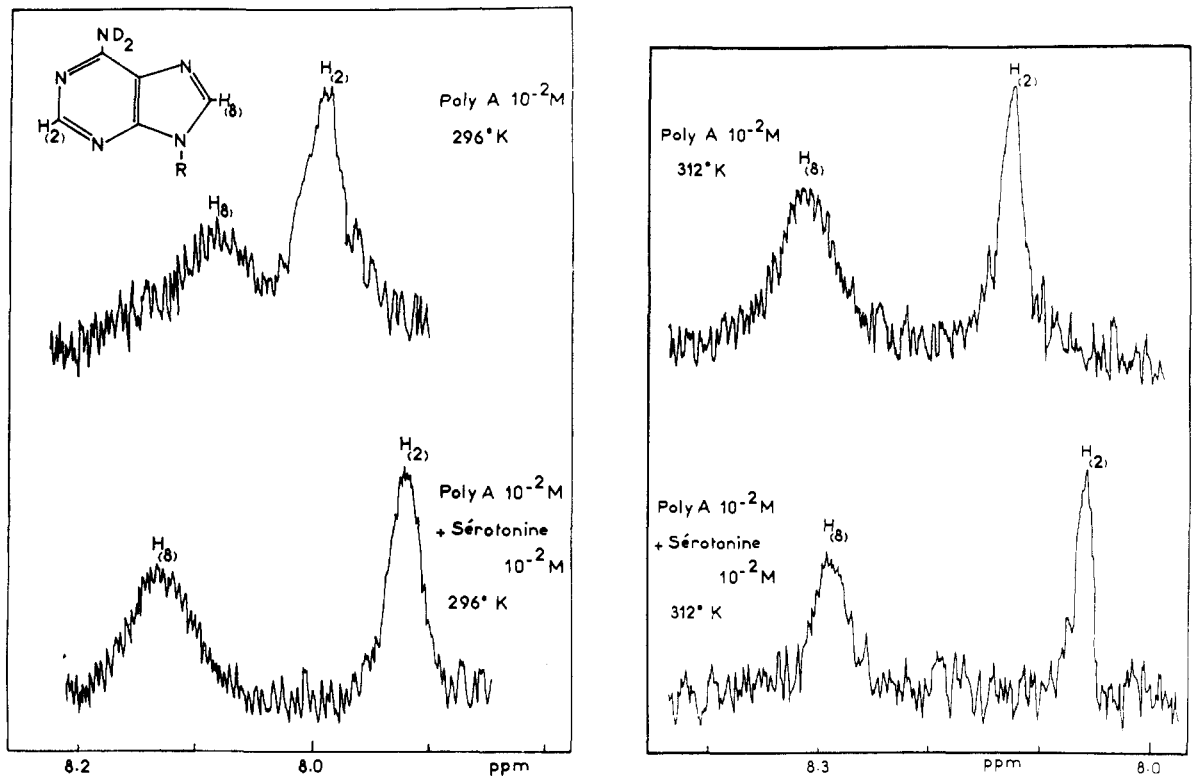

FIGURE 11: Resonances of the H_8 and H_2 protons of adenine in poly(A) (10^{-2} M) at pD 7 in the absence (upper spectrum) and the presence (lower spectrum) of serotonin (10^{-2} M). (a, left) 296°K and (b, right) 312°K.

indole ring and the purine and pyrimidine bases in stacked complexes. Other observations such as the increase in stability of DNA upon binding of tryptophan-containing dipeptides are also in agreement with such a stacking interaction (Brown, 1970). One cannot eliminate the possibility of hydrogen bonding between the NH group of the indole ring and the bases. In the case of serotonin, the hydroxyl group does not appear to be involved in hydrogen bonding since 5-methoxytryptamine binds equally well to DNA. All the experimental results available favor binding of the intercalation type. Tryptamine and serotonin bind almost as well to single-stranded polynucleotides or denatured DNA as to native double-stranded DNA. This suggests that the indole ring should interact with the bases of only one of the DNA chains. This is expected since the size of the indole ring is quite similar to that of the purine ring. Model building studies show that such a location of the indole ring is compatible with the electrostatic interaction described above. Proton magnetic resonance studies of serotonin–poly(A) complexes also demonstrate that serotonin intercalates between adenine bases and that the indole rings are stacked with the purine rings. Although this conclusion cannot be extrapolated without caution from single-stranded poly(A) to double-stranded DNA, the similarity of fluorescence quenching results provides supporting evidence for a stacking interaction between the indole ring of tryptamine or serotonin and the DNA bases. Changes in the circular dichroism spectrum of DNA that are observed upon binding of tryptamine or serotonin also indicate a perturbation of the DNA conformation (H. A. Borazan, M. Durand, and C. Hélène, to be published).

This type of interaction between the indole ring of tryptophan residues and nucleic acid bases could play an important role in the binding of proteins or enzymes to nucleic acids. Fluorescence studies demonstrate that the aromatic ring of tyrosine is also able to interact with nucleic acid bases (Montenay-Garestier and Hélène, 1970; Hélène, 1971). Therefore, these aromatic amino acid residues could intercalate between the bases of a nucleic acid, thus constraining the nucleic acid molecule to adopt a definite position with respect to the enzyme molecule. The presence of aromatic amino acid residues in the active site of nucleases has been demonstrated (Cuatrecasas *et al.*, 1968; Pongs, 1970). However it must be emphasized that the specificity of recognition of a nucleic acid by an enzyme might involve a number of subsites different from the active site. The existence of enzyme subsites which recognize bases several positions removed from the active center has been suggested to explain the specific fragmentation of ribonucleic acids by nucleases (Pinder and Gratzer, 1970). The distribution of the aromatic amino acid residues in the enzyme structure could ensure the correct positioning of the nucleic acid molecule that would be necessary for further reactions. The secondary structure of poly(A) and DNA is affected by the binding of serotonin and tryptamine. This conformational change could also be required for enzymatic action. In the particular case of tRNA binding to aa-tRNA synthetases, we have shown for the valine system from *Escherichia coli* that the fluorescence of tryptophan residues of the synthetase is quenched by $tRNA^{Val}$ (Hélène *et al.*, 1969, 1971; Hélène, 1970). Similar quenching effects were observed more recently in the case of methionine (Bruton and Hartley, 1970) and phenylalanine (Farrelly *et al.*, 1971) from *E. coli* and serine from yeast (Rigler *et al.*, 1970). Different mechanisms have been put forward to explain this result (Hélène *et al.*, 1971). The observations reported here which

show that stacking interaction between the indole ring of tryptophan and the bases is accompanied by fluorescence quenching provide a plausible explanation.

The molecular characteristics of the serotonin receptor site in the brain have not been as yet elucidated. Although it was generally assumed that the receptor site for transmitters in neuronal membrane must be constituted by proteins, the discovery of RNA in membrane suggested that the receptor site could be RNA or a complex of RNA with proteins and prostaglandins (Smythies, 1970). The results obtained by Smythies and Antun (1969) and those presented here provide experimental evidence for stacking interactions between serotonin and nucleic acids. Hallucinogenic drugs whose chemical structure is related to tryptamine or serotonin such a dimethyltryptamine, harmine, psilocine, psilocybine, and lysergic acid diethylamide seem to act primarily on the central serotonin mechanism (Smythies, 1970). In one case at least (lysergic acid diethylamide), interaction with double-stranded DNA has been demonstrated (Yielding and Sterglanz, 1968). Thus the site of action of these hallucinogenic drugs could also be constituted by nucleic acids.

The study of interactions between amino acids or their derivatives (including oligopeptides) and the components of nucleic acids or nucleic acids themselves should provide informations on the different mechanisms by which the amino acid residues of enzymes or proteins are involved in the recognition of nucleic acids. Once these different types of interactions have been elucidated, the specificity of recognition could be understood not only on the basis of stereochemical relationships but also from the sequence of amino acids or bases in the recognition sites. Studies along this line are currently in progress in our laboratory.

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