# Reflectance and Luminescence Studies of Molecular Complex Formation between Tryptophan and Nucleic Acid Components in Frozen Aqueous Solutions*

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ABSTRACT: Reflectance and luminescence measurements are used to show that tryptophan forms molecular complexes with nucleosides in the aggregates that form when aqueous solutions are frozen. A new absorption appears at longer wavelengths than the absorption of the components and there is a new fluorescence band characteristic of the complex. The variation of absorption intensity with tryptophan concentration at a fixed nucleoside concentration allows us to determine the stoichiometry of the complex. At neutral pH, the formation of a 1:1 complex is observed. In acid medium, when the nucleosides cytidine and adenosine bear a positive charge their complexes have a 1:2 (nucleoside to tryptophan) stoichiometry. The first excited singlet state of the complex has an energy lower than that of the two isolated components and can thus behave as a trap for excitation energy migrating in the aggregates formed in frozen aqueous solutions. Transfer of charge from the indole ring of tryptophan to the purine or pyrimidine ring of the nucleoside is shown to be involved in complex formation. Pyrimidines are found to be better electron acceptors than purines.

Most of the fundamental steps involved in the expression of the cellular genome require formation of specific complexes between nucleic acids and proteins or enzymes. The specificity of the recognition process could be due to specific interactions between a few bases of the nucleic acid and a few amino acid residues in the protein or the enzyme.

A number of studies has been performed on complexes of polynucleotides or DNA with basic polypeptides (see, for example, Leng and Felsenfeld, 1966, Sober *et al.*, 1966, Olins *et al.*, 1967, Shapiro *et al.*, 1969, and references cited therein). Interactions between mononucleotides and basic polypeptides (Woese, 1968; Wagner and Arav, 1968) and between amino acid derivatives and polynucleotides (Gabbay and Kleinman, 1967) have also been demonstrated. These studies have led to the conclusion that some specificity does exist although its origin is not elucidated as yet. A systematic study of interactions between amino acids and nucleic acid components should shed some light on this problem. These interactions should modify the spectroscopic properties of one of the interacting species or of both. The observed absence of interactions in dilute aqueous solutions could have its origin in the fact that these molecules are strongly solvated. However, the situation might be quite different in enzyme–nucleic acid interactions since the structure of the solvent in the active site, and therefore the solvation of its amino acid residues, may have nothing in common with that observed in an aqueous solution of the same residues. Moreover, nucleic acid bases can be brought into the active site of enzymes by strong nonspecific electrostatic forces. Then they can have specific interactions with the amino acids residues of the active site. Close contact between molecules can be induced by freezing down aqueous solutions. During the freezing of an aqueous solution, solute molecules are excluded from the growing ice crystals and accumulate in cavities where they form aggregates. The structure of these aggregates is very likely similar to that of microcrystals. Many phenomena, such as thymine photodimerization in ice (Wang, 1965), dipolar broadening of electron spin resonance spectra of paramagnetic metal cations (Ross, 1965), and enhancement of bimolecular reaction rates upon freezing aqueous solutions (Bruice and Butler, 1965), have been already ascribed to the formation of aggregates. We have investigated aggregates of nucleosides as models for energy-transfer studies (Hélène, 1966; Hélène and Montenay-Garestier, 1968). When an aqueous solution containing two kinds of solute molecules is frozen down, mixed aggregates can form. Energy transfer at the triplet level can be readily demonstrated in these mixed aggregates. Such a transfer occurs by an exchange mechanism and involves overlapping of electron clouds of the energy donor and acceptor, thereby proving that stacked structures do exist in these aggregates (Hélène and Montenay-Garestier, 1968). We have used these aggregates to demonstrate the possibility of molecular interactions between amino acids and nucleic acid components. We report here the results of a study of such interactions between tryptophan (and several related compounds) and bases or nucleosides. A preliminary account of this work has been already published (Montenay-Garestier and Hélène, 1968). Because of the microcrystalline state of the samples, the absorption spectra of the frozen aqueous solutions cannot be measured by standard transmission techniques. We have deduced these absorption spectra from the reflectance spectra of our frozen samples.

## Experimental Section

*Compounds.* Most of the compounds used in this study (tryptophan and nucleosides) were purchased from California Corp. for Biochemical Research. Substituted tryptophan derivatives were obtained from Cyclo Chemical Corp. Solutions were made with bidistilled water and the pH adjusted

* From the Centre de Biophysique Moléculaire, La Source, 45-Orléans-02, France. *Received July 14, 1970.* This is part II of the series: Molecular Interactions between Amino Acids and Nucleic Acid Components. For Part I, see Montenay-Garestier and Hélène (1968).

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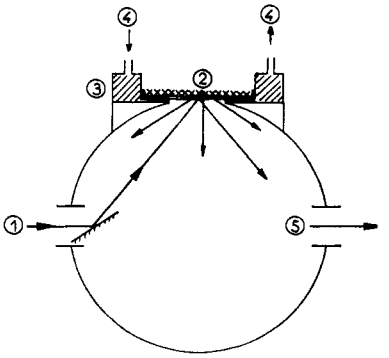


FIGURE 1: Schematic drawing of the experimental arrangement used to measure reflectance spectra (1) excitation beam, (2) quartz cell containing the frozen sample, (3) copper block used to cool the cell, (4) circulation of refrigerated methanol, and (5) exit aperture.

using a Tacussel pH-titrimeter TS 40/N at room temperature.

*Luminescence Measurements.* Fluorescence and phosphorescence measurements were carried out with a Jobin-Yvon spectrofluorimeter equipped with two quartz-prism monochromators, an Osram XBO 250-W lamp and a 1P28 photomultiplier. For room-temperature measurements, the solution was contained in a 1-cm quartz cell with viewing of the fluorescence from the front face. For low-temperature measurements at liquid nitrogen temperature (77°K), the frozen solution was contained in a 2-mm i.d. quartz tube which could be set to the position of maximum emission intensity by a mechanical device. To clearly separate fluorescence from scattered light, filters were inserted in the excitation beam. Phosphorescence was separated from fluorescence with a rotating can phosphoroscope. Luminescence and phosphorescence spectra are not corrected to take account of photomultiplier sensitivity or monochromator transmission.

*Reflectance Measurements.* The total diffuse reflectance accessory of the Cary 14 spectrophotometer was adapted for measurements at temperatures between −40 and 0° as described in Figure 1. The temperature was kept constant within ±1° by circulating refrigerated methanol. Measurements were made relative to a white MgO standard for which it is assumed that absorbance is 0 in the spectral region investigated and that the percentage of incident light reflected does not depend on the wavelength.

Absorption spectra were deduced from reflectance spectra in the following way. Let $I_1$ be the intensity of light scattered by ice when there is no absorption of light (no solute), $I_2$ the intensity of light scattered by a frozen sample which absorbs all the incident light, and $I$ the intensity of light scattered by the frozen aqueous solution under study. (In each case, the measured intensity is in fact the sum of scattered light and light reflected by the quartz plate.) The percentage of light intensity scattered by the sample is

$$R = \frac{I - I_2}{I_1 - I_2} \qquad (1)$$

For infinite layer thickness, $R$ is related to the extinction coefficient, $\epsilon$, and the scattering coefficient, $s$, by the Kubelka–Munk relation (Wendlandt and Hecht, 1966).

$$\frac{(1 - R)^2}{2R} = \frac{2.3\epsilon c}{s} \qquad (2)$$

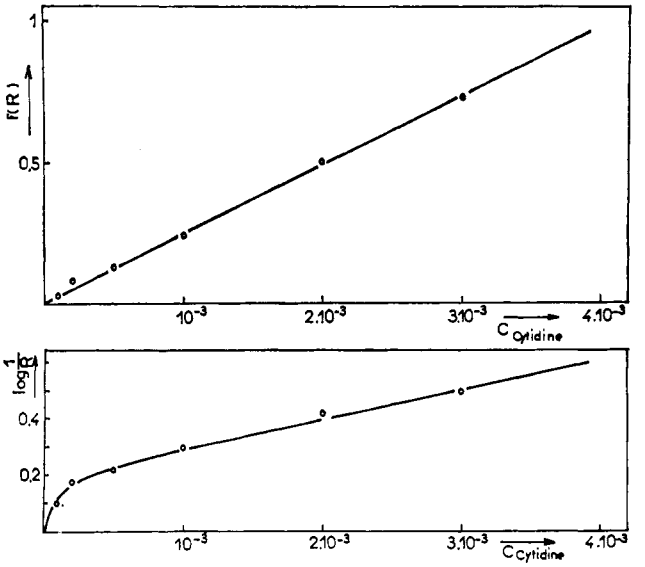


FIGURE 2: Dependence on cytidine concentration of the functions f($R$) and log (1/$R$), where $R$ represents the percentage of light scattered by the sample at 300 nm in ice at −20°, pH 7 (see text for explanations).

The function on the left-hand side of eq 2 is called the remission function or the Kubelka–Munk function, and this will be noted as f($R$). The scattering coefficient, $s$, is not known but it is assumed to be independent of wavelength because the scattering particles (ice microcrystals) are large in comparison with the wavelength of the radiations involved.

The derivation leading to eq 2 requires that the layer have an infinite thickness. No difference in reflectance spectra could be observed in our experimental arrangement when the thickness was more than about 2 mm. All our experiments were performed with layers of 3 mm thickness. Equation 2 was used to calculate the function f($R$). Transmission spectroscopy defines the optical density of the sample by log (1/$R$). This quantity was also calculated and was compared to f($R$).

In Figure 2, we show the dependence of f($R$) and log (1/$R$) at 300 nm on the cytidine concentration in ice at −20°. It can be seen that f($R$) depends linearly on cytidine concentration. This is not the case with log (1/$R$) at least for $c < 10^{-3}$ M. From the slope of the straight line obtained when plotting f($R$) *vs.* $c$, $s$ can be calculated. At 300 nm for cytidine, a value of 1.4 has been obtained. If one assumes that $s$ does not vary with wavelength, then $\epsilon$ of a frozen sample can be obtained by: $\epsilon = sf(R)/2.3c$.

Tryptophan in frozen aqueous solutions has a high fluorescence quantum yield so that the observed reflectance spectrum is not directly related to its absorption spectrum. However, reflectance measurements of the complexes have always been carried out at wavelengths where tryptophan does not absorb light. Moreover, we have generally used a nucleoside concentration higher than that of tryptophan so that all tryptophan molecules are complexed and then they have a very low fluorescence quantum yield (see below).

## Results

*Complex Formation between* L-*Tryptophan and Nucleosides in Frozen Aqueous Solutions at Neutral pH.* When an aqueous solution at pH 7, containing an equimolar mixture of L-tryptophan and one of the nucleosides (guanosine, adenosine,

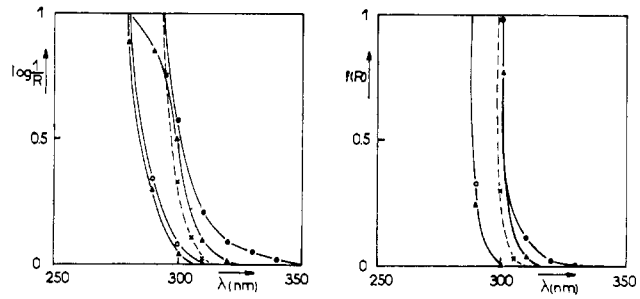

FIGURE 3: Plots of the functions $f(R)$ and $\log (1/R)$ *vs.* wavelength for frozen aqueous solutions $10^{-3}$ M (pH 7) of adenosine (△), uridine, (○) tryptophan (×), and the equimolar ($10^{-3}$ M) mixtures adenosine + tryptophan (▲) and uridine + tryptophan (●).

uridine, thymidine, and cytidine) is frozen, a new absorption and a new fluorescence appear to wavelengths longer than those of the two components (Figures 3, 4a, and 5a) (Table I). This indicates that molecular interactions do occur in the equimolar mixture. These effects are much more important with pyrimidines than with purines (Figure 3 and Table I).

The absorbance of the frozen sample at −20° depends on the nucleoside and on the relative concentrations of both tryptophan and the nucleoside as is shown in Figure 6. When

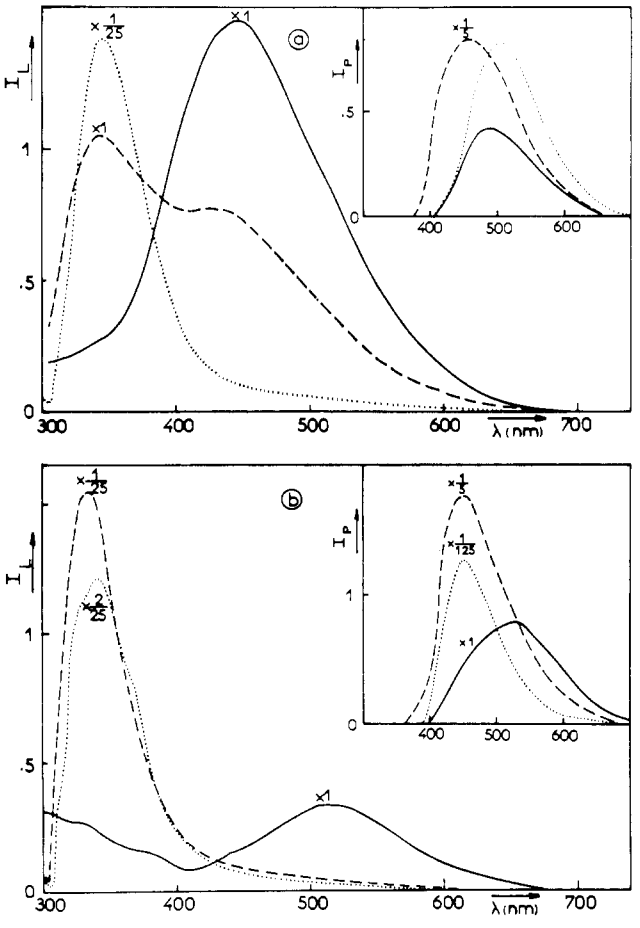

FIGURE 4: Luminescence and phosphorescence spectra (insert) of $2 \times 10^{-3}$ M aqueous frozen solutions at 77°K of tryptophan (· · ·), cytidine (– – –), and their equimolecular mixture ($2 \times 10^{-3}$ M each) (——). (a) pH 7 and (b) pH 2. Intensities in arbitrary units are multiplied by the numbers indicated on the spectra. Excitation wavelength is at 280 nm.

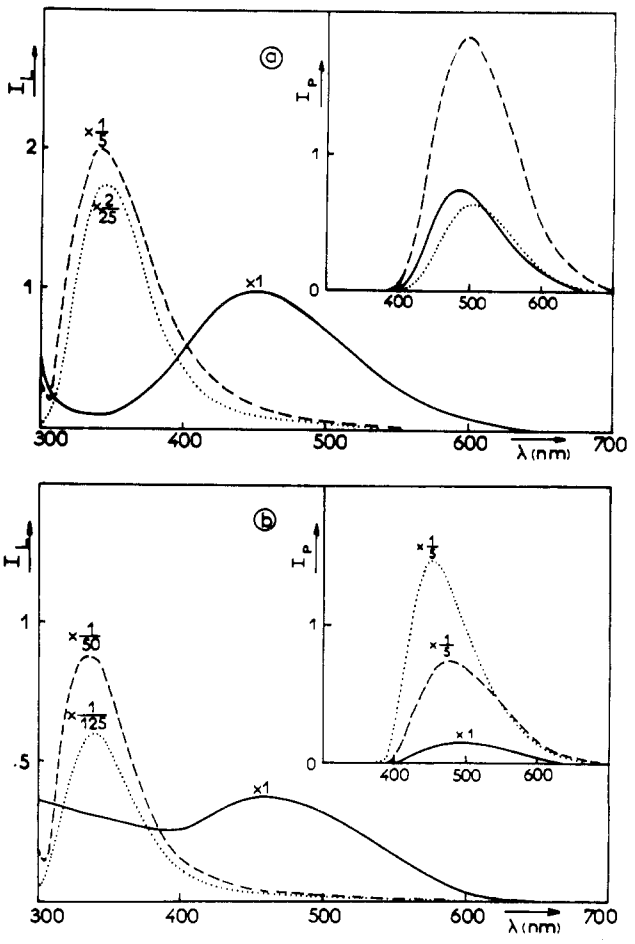

FIGURE 5: Luminescence and phosphorescence spectra (insert) of $2 \times 10^{-3}$ M aqueous frozen solutions at 77°K of tryptophan (· · ·), thymidine (– – –), and their equimolecular mixture (——). (a) pH 7 and (b) pH 2. Excitation wavelength is at 280 nm.

the nucleoside concentration is kept constant ($10^{-3}$ M in most of our experiments) and tryptophan concentration is increased, the optical density at a given wavelength reaches a plateau, when the concentration ratio is about 1 (whatever the nucleoside). This implies that complex formation with a 1:1 stoichiometry is observed. The reflectance spectra extend to much longer wavelengths with pyrimidine complexes than with purine complexes (Figure 3). When $f(R)$ is plotted against the ratio of tryptophan and nucleoside concentrations, the slope at the beginning of the curve is lower than is expected from the remainder of the curve when only one complex is considered to form (Figure 6a, see also Figure 11). This deviation could be attributed to the concomitant formation of a complex with a different stoichiometry. Since the nucleoside has the highest concentration, this complex would probably be 2:1 (nucleoside:tryptophan). When $\log (1/R)$ is plotted *vs.* $r$, the previous deviation is not observed. This is expected since $\log (1/R)$ is not subject to a linear increase with concentration (see Figure 2). Though the plateau which is observed with $f(R)$ is also observed with $\log (1/R)$.

The fluorescence spectra of equimolar mixtures of tryptophan and nucleosides have their maxima at longer wavelengths with pyrimidines than they do with purines, which is in agreement with the reflectance results (Table I). The fluorescence intensity of the equimolar mixture is much lower than is observed from free tryptophan. The quenching is

TABLE I: Wavelengths of the Fluorescence Maximum of Tryptophan and Nucleosides in Frozen Aqueous Solutions (Left Column) and of Equimolecular Mixture of Tryptophan and Nucleosides at 77°K ($2 \times 10^{-3}$ M Each) (Right Column).

| Fluorescence, $\lambda_{max}$ (nm) (Aggregates)[a] | Compound | Fluorescence, $\lambda_{max}$ (nm), of Equimolecular Mixtures of Nucleosides with Trp (Mixed Aggregates) |
|---|---|---|
| 342 | Tryptophan | |
| 352 | Guanosine | 355 |
| 348 | Adenosine | 380 |
| 345 | Cytidine | 450 |
| ~335 | Uridine | 455 |
| 340 | Thymidine | 450 |
| 325 | Adenosine cation | No fluorescence[b] |
| 332 | Cytidine cation | No fluorescence[b] |

[a] This represents the wavelength of the fluorescence band maximum of each compound (listed in column 2) in a frozen aqueous solution $2 \times 10^{-3}$ at 77°K. [b] Mixed aggregates of tryptophan and adenosine or cytidine at pH 2 emit only phosphorescence ($\lambda_{max}$ 500 nm).

greater with pyrimidines than it is with purines. The red shift to the fluorescence spectrum indicates that the lowest excited singlet state of the complex has an energy lower than that of the two individual components. When the ratio of tryptophan and nucleoside concentration is different from unity, energy absorbed by molecules of the component in excess can migrate from one molecule to the other and can be ultimately trapped by the complex or else it can be transferred directly to the complex by the Förster long-range mechanism. Fluorescence measurements cannot therefore be used to determine the stoichiometry of the complex. Figure 7a shows the fluorescence quenching of adenosine and thymidine by tryptophan. Figure 7b shows the fluorescence quenching of tryptophan by adenosine and thymidine. The probability for singlet energy transfer by Förster's mechanism increases when the overlap of the fluorescence spectrum of the donor and the absorption spectrum of the acceptor increases (Förster, 1965). In accord with the previous reflectance studies, tryptophan and thymidine transfer their excitation energy to the complex tryptophan–thymidine with a higher efficiency than tryptophan and adenosine do to their complex tryptophan–adenosine. Nevertheless, the phosphorescence of an equimolar mixture of tryptophan and a nucleoside is quite similar to that of tryptophan alone (Figures 4 and 5).

If the fluorescence of $10^{-3}$ M aqueous solution of tryptophan at 20° is measured as a function of nucleoside concentration, the fluorescence intensity decreases. This decrease can be entirely accounted for by the screening effect of the nucleoside. The measurements are carried out in a 1-cm quartz cell with front face viewing of the fluorescence. All the incident light intensity, $I_0$, at the excitation wavelength (290 nm) is absorbed by the sample. If $d_0$ represents the optical density of tryptophan and $d$ that of the nucleoside (nonfluorescent) the part of light intensity absorbed by tryptophan is

$$I = I_0 \frac{d_0}{d + d_0} \qquad (3)$$

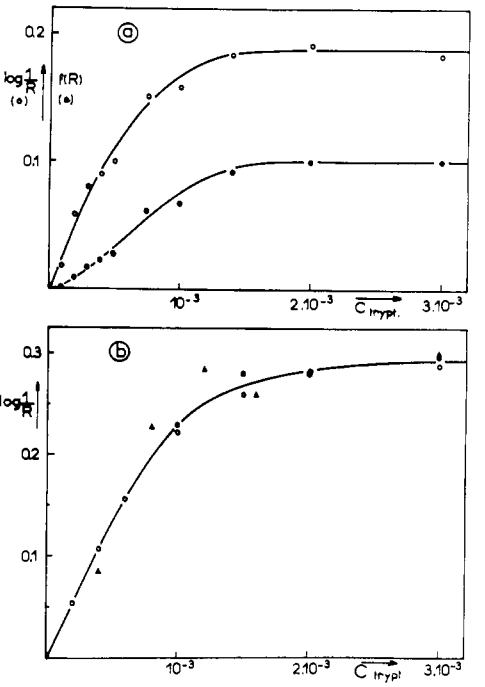


FIGURE 6: Dependence on tryptophan concentration. (a) Of the functions log (1/$R$) (○) and f($R$) (●) at 320 nm for frozen aqueous solutions containing uridine $10^{-3}$ M. (b) Of the function log (1/$R$) at 320 nm for frozen aqueous solutions containing cytidine $10^{-3}$ M (○) or cytidine $10^{-3}$ M + glycine $10^{-3}$ M (△). The filled circles represent values of log (1/$R$) obtained with a constant concentration of tryptophan ($10^{-3}$ M) and different concentrations of cytidine (same abscissa scale).

Let $I_F^0$ and $I_F$ represent the fluorescence intensities of tryptophan in the absence and the presence of the nucleoside (optical density, $d$). The corresponding fluorescence quantum yields of tryptophan are

$$\rho_F^0 = \frac{I_F^0}{I_0} \qquad \rho_F = \frac{I_F}{I}$$

Therefore

$$\frac{\rho_F}{\rho_F^0} = \frac{I_F}{I_F^0} \frac{d + d_0}{d_0}$$

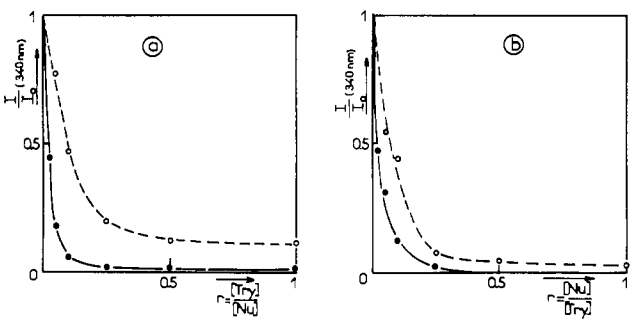


FIGURE 7: Variation of the relative fluorescence intensity $I/I_0$ at 340 nm with the ratio $r$ of tryptophan (Trp) and nucleoside (Nu) concentrations. Excitation wavelength is at 280 nm. (a) Nucleoside concentration is kept constant ($10^{-3}$ M) and tryptophan concentration increases. (○) Adenosine and (●) thymidine. (b) Tryptophan concentration is kept constant ($10^{-3}$ M) and nucleoside concentration increases. (○) Adenosine and (●) thymidine.

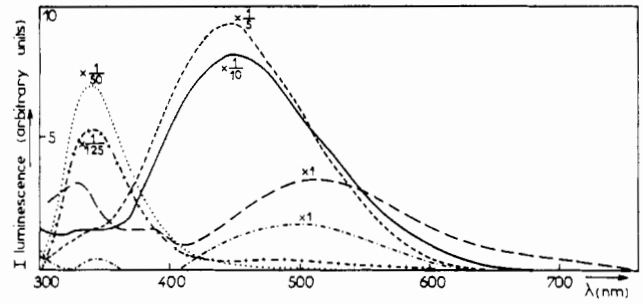


FIGURE 8: Luminescence spectra of $2 \times 10^{-3}$ M frozen aqueous solutions at 77°K of L-tryptophan methyl ester, TME (···); *N*-acetyl-L-tryptophanamide, ATA (×-×-×); and their equimolecular mixtures with cytidine, C. Excitation wavelength is at 280 nm. (——) TME + C, pH 7; (– –) TME + C, pH 2, (- - -) ATA + C, pH 7; and (–·–·–) ATA + C, pH 2.

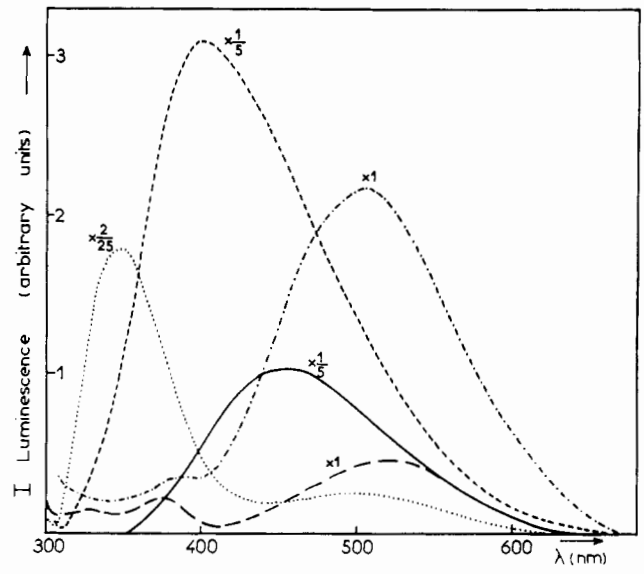


FIGURE 9: Luminescence spectra of $3 \times 10^{-3}$ M frozen aqueous solutions at 77°K of tryptamine (···) and equimolecular mixtures of tryptamine and adenosine ($3 \times 10^{-3}$ each) at pH 6.5 (– – –) and pH 2.2 (–·–·–) and equimolecular mixtures of tryptamine and cytidine ($3 \times 10^{-3}$ M each) at pH 6.5 (——) and 2.2 (— —).

With all nucleosides tested $\rho_F/\rho_F^0$ remains unity when $d$ increases. Therefore no complex formation is observed under our experimental conditions in fluid dilute aqueous solutions (for experimental results concerning formation in concentrated aqueous solution, see part III in this series, C. Hélène *et al.*, in preparation).

*Complex Formation between Tryptophan Derivatives and Nucleosides.* To determine the respective role of the indole ring and the amino acid chain in the process of complex formation, we have investigated a number of derivatives in which the carboxylic or amino group has been blocked or eliminated. In frozen aqueous solutions, at pH 7, indoleacetic acid, tryptamine, D-tryptophan, L-tryptophan methyl ester, *N*-acetyl-L-tryptophan, and *N*-acetyl-L-tryptophanamide give fluorescence and reflectance results similar to those obtained with tryptophan. Some of the spectra are shown in Figures 8 and 9. The reflectance spectra of the complexes only slightly depend on the nature of the substituents. The dipeptides glycyl-L-tryptophan and L-tryptophylglycine and the tripeptide glycyl-L-tryptophylglycine behave the same way. Only indole itself and *N*-acetyl-L-tryptophan methyl ester do not provide any evidence for complex formation. However these compounds also have a very poor solubility in water. Complex formation in a frozen aqueous solution is due to the formation of mixed aggregates during the freezing of the aqueous mixture (see Discussion). A poor solubility in water implies a tendency to self-aggregation which is thought to prevent mixed aggregate formation. Formation of separate aggregates for weakly interacting systems has been already observed. For example, the color due to weak charge-transfer complexes in the liquid phase frequently disappears when the mixture is frozen down because microcrystals of both donor and acceptor form separately (Hammond and Burkardt, 1968, 1970).

Only small differences are seen when the nucleoside is replaced by the corresponding base or nucleotide. This indicates that neither the ribose nor the phosphate are required to observe the interaction with tryptophan.

If the amino acid chain is involved in the interaction of tryptophan and nucleosides, an aliphatic amino acid such as glycine should be able to compete with tryptophan. Figure 6b shows that glycine at a concentration of $10^{-3}$ M has no effect on the reflectance intensity of the complex in mixtures of cytidine ($10^{-3}$ M) and tryptophan, when tryptophan concentrations increases from 0 to $2 \times 10^{-3}$ M. Therefore glycine does not prevent complex formation.

All the results are thus consistent with the involvement of the indole ring of tryptophan and the base moiety of the nucleoside in the formation and the spectroscopic properties of the complex.

*pH Dependence of Complex Formation between Tryptophan and Nucleosides.* Since the degree of ionization of nucleic acid bases can be varied by changing the pH of the solution, we have studied complex formation between tryptophan and nucleosides in different ionization states.

The pH of the solutions is measured at room temperature. The value of the pH at the time of aggregate formation during the freezing of the solution might be different from this measured value. However, the p$K$ value of cytidine deduced from fluorescence and phosphorescence measurements in aggregates, using room-temperature values of the pH, is nearly identical with the p$K$ determined in fluid aqueous solution (Montenay-Garestier and Hélène, 1970). It is generally assumed that the pH dependence of fluorescence and phosphorescence in rigid medium follows the ground-state protonation equilibrium because excited-state lifetimes are short (especially the fluorescent singlet state) as compared to the time required to shift the protonation equilibrium (Montenay-Garestier and Hélène, 1970). Therefore, the protonation state in the aggregates of the solute molecules studied here should not be very different from that deduced from room-temperature measurements of the pH.

The red shift of the luminescence spectrum of the mixtures tryptophan–nucleoside as compared to those of the two components is more important when the base bears a positive charge as in the case for cytidine or adenosine in acid medium (Figure 4b). This effect is not observed in acid medium with thymidine which cannot bind a proton (Figure 5b). The $\lambda_{max}$ of the luminescence spectrum of the equimolar mixture cytidine and tryptophan shifts from 450 nm at pH 7 to 495 nm at pH 2. The pH dependence of this luminescence $\lambda_{max}$ is shown in Figure 10. From the curve thus obtained, a p$K$ of about 4.3 can be calculated. This value is quite close to the p$K$ of cytidine in frozen aqueous solution as deduced from

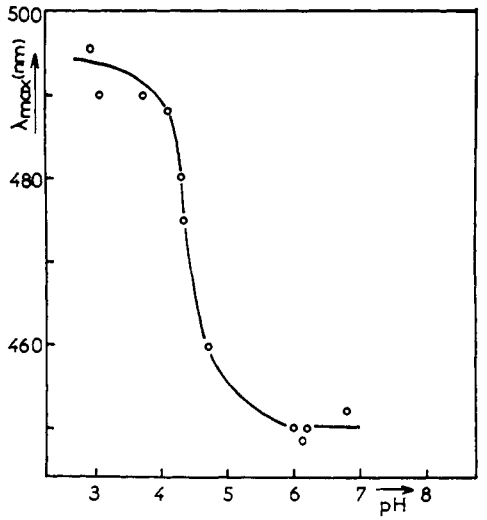


FIGURE 10: pH dependence of the luminescence maximum of an equimolecular mixture of tryptophan and cytidine ($5 \times 10^{-3}$ M each) in frozen aqueous solutions at 77°K. pH values are measured at room temperature. Excitation wavelength is at 280 nm.

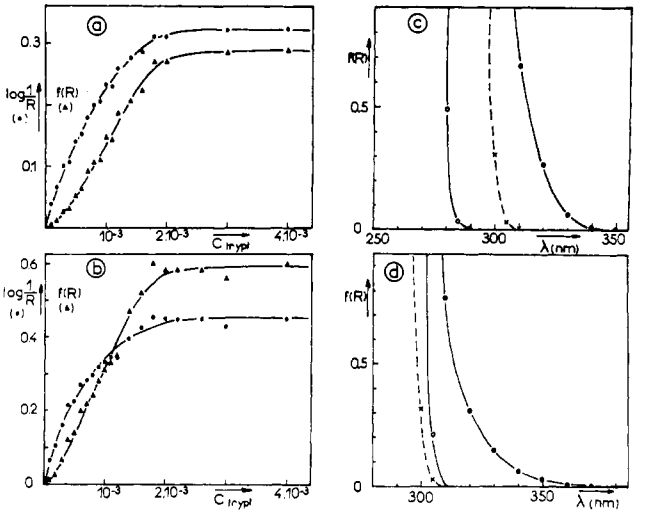


FIGURE 11: (a and b) Dependence of the functions f($R$) and log ($1/R$) at 320 nm on tryptophan concentration for frozen aqueous solutions at −20° containing adenosine $10^{-3}$ M (a) or cytidine $10^{-3}$ M (b) at pH 2. (c and d) Wavelength dependence of the function f($R$) for frozen aqueous solutions at −20°, pH 2. (c) Adenosine, $10^{-3}$ M (○); tryptophan, $10^{-3}$ M (×); and adenosine + tryptophan ($10^{-3}$ M each) (●). (d) Cytidine, $10^{-3}$ M (○); tryptophan, $10^{-3}$ M (×); and their equimolecular mixture ($10^{-3}$ M each) (●).

luminescence titration curves (Montenay-Garestier and Hélène, 1970). Protonation of cytidine is thus demonstrated to cause the observed effects. Similar results are obtained with other indole derivatives. Figure 8 shows that tryptophan methyl ester and *N*-acetyl-L-tryptophanamide, in equimolar mixtures with cytidine, give identical results, either at pH 7 or pH 2. The pH dependence of the luminescence characteristics of equimolar mixtures of tryptamine and cytidine or adenosine is described in Figure 9. The luminescence of these complexes in acid medium appears to be only phosphorescence. This phosphorescence has its maximum at longer wavelengths than the phosphorescence of the individual components and thus appears to be characteristic of the complexes.

The reflectance spectrum of the mixtures of cytidine (or adenosine) and tryptophan extends to much longer wavelengths at pH 2 than at pH 7 in agreement with fluorescence results. Again no change is observed in the reflectance spectrum of the mixture of uridine (or thymidine) and tryptophan between pH 2 and 8 because uridine (or thymidine) remains un-ionized in this pH range. A study of the dependence of the reflectance intensity at a given wavelength *vs.* the ratio $r$ of tryptophan and nucleoside concentration indicates that a plateau is reached when $r > 2$ (Figure 11). The main complex that is formed in acid medium with cytidine and adenosine should therefore have a 2:1 stoichiometry (tryptophan:nucleoside). As already noticed at pH 7, some curvature is also observed at pH 2 when f($R$) is plotted *vs.* $r$. This could indicate the intermediate formation of a 1:1 complex. It should be noted that in fluid acid aqueous solutions, a 1:1 composition is obtained for the complexes tryptophan–cytidine and tryptophan–adenosine as determined from Job diagrams (see next paper in this series, C. Hélène *et al.*, in preparation) although the absorption spectrum is very similar to the reflectance spectrum. If the complex really has a 2:1 stoichiometry at pH 2, it should have a sandwich structure: one protonated base between two tryptophan molecules. But this sandwich structure could also be due to electrostatic repulsion between protonated nucleoside molecules that prevent them to come too close together. This problem is similar, for example, to the electrostatic repulsion between cationic dyes intercalated in double-stranded DNA (Gilbert and Claverie, 1968). It is therefore possible that the true stoichiometry of the complex tryptophan–nucleoside is 1:1 at pH 2 as well as at pH 7 although its maximum formation is achieved only when two nucleoside molecules are separated by two tryptophan molecules.

In alkaline medium (NaOH, $10^{-2}$ M), cytidine still quenches the fluorescence of tryptophan as observed in neutral medium. However, the interaction between tryptophan and uridine is not observed in alkaline medium. These results are expected if charge transfer plays an important role in complex formation (see below). Uridine that has lost a proton in alkaline medium becomes negatively charged and probably can no longer accept an electron. Cytidine that cannot lose a proton gives similar results in neutral and alkaline medium.

## Discussion and Conclusion

The results reported above show that molecular interactions between tryptophan (or its derivatives) and nucleic acid components can be observed in frozen aqueous solutions. As pointed out in the introduction, freezing an aqueous solution induces the formation of aggregates of solute molecules excluded from the growing ice crystals. In mixed aggregates formed upon freezing an aqueous mixture of two water-soluble compounds, different molecules can thus be forced to interact although interaction with solvent molecules is strong enough to prevent complex formation in the liquid phase. At low temperature (−150°) in a mixture of methanol and water, Hui Bon Hoa and Douzou (1970) have shown that tryptophan and uridine could form a complex whose fluorescence is similar to that reported in this study. The stoichiometry of the complexes studied here is 1:1 when the base is in its neutral state whereas it seems to be 1:2 (nucleoside:tryptophan) when the base is protonated in acid medium.

Reflectance spectra of the complexes indicate the presence

of a new absorption, without well-defined maximum at wavelengths longer than those of tryptophan and nucleoside absorption. The appearance of a new fluorescence spectrum also confirms that the complex possesses a singlet state of energy lower than those of the components. This behavior is characteristic of weak charge-transfer or electron donor–acceptor complexes (Mataga and Murata, 1969). In the ground state, the stabilizing forces are mainly van der Waals–London electrostatic forces. Transfer of electronic charge occurs upon excitation. Charge-transfer contribution is thus enhanced in the excited state of the complex. The fluorescence spectrum is therefore expected to be much more affected than the absorption spectrum (Mataga and Murata, 1969).

The shift of the fluorescence maximum depends on the extent of electron transfer from the donor to the acceptor. Indole derivatives are known to be good electron donors and form electron donor–acceptor complexes with several electron acceptors (Foster and Hanson, 1964; Foster and Fyfe, 1966). In the complexes described above, the indole ring of tryptophan acts as an electron donor. Molecular complexes involving tryptophan have also seen described (Birks and Slifkin, 1963; Wilson, 1966; Pereira and Tollin, 1967). From the value of the fluorescence, $\lambda_{max}$ (Table I), and from absorption spectra (Figures 3 and 11), one can deduce that electron affinities of the nucleosides increase in the order guanosine < adenosine < cytidine, uridine, thymidine < adenosine cation < cytidine cation. This represents of course a property of the base moiety (and not the ribose) of the nucleoside. For unprotonated bases, this order is in agreement with that deduced from the energy of the lowest empty molecular orbital calculated by SCF methods (Berthod *et al.*, 1966). Protonated bases should be better electron acceptors than the neutral bases due to the self-consistent field positive charge. This is experimentally observed. It should be noticed that an electron donor–acceptor complex between cytidine and its cation has been already described (Montenay-Garestier and Hélène, 1970).

The formation of electron donor–acceptor complexes between tryptophan and nucleosides could play an important role in the interactions between proteins or enzymes and nucleic acids. For example, the quenching of tryptophan fluorescence due to the binding of tRNA to valyl-tRNA synthetase (Hélène *et al.*, 1967) could be interpreted on this basis (although other explanations, such as conformational change, are, of course, possible). Studies of interactions between other amino acids or oligopeptides and nucleosides or DNA itself should help understand the nature and specificity of interactions between enzymes (or proteins) and nucleic acids. They are presently in progress in our laboratory.

Acknowledgments

We thank Professor Ch. Sadron for his interest in this work and Miss F. Brun for her skillful assistance in measuring reflectance spectra.

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