Shapiro, H. S. (1968), *in* Handbook of Biochemistry, Sober, H. A., Ed., Cleveland, Ohio, Chemical Rubber Co., p H-30.
Shapiro, R., Servis, R. E., and Welcher, M. (1970), *J. Am. Chem. Soc. 92*, 422.
Smith, K. C., and Aplin, R. T. (1966), *Biochemistry 5*, 2125.
Wang, S. Y. (1957), *Nature 180*, 91.
Wang, S. Y. (1958), *J. Am. Chem. Soc. 80*, 6196.
Wang, S. Y. (1959), *J. Org. Chem. 24*, 11.
Wang, S. Y., Apicella, M., Stone, B. R. (1956), *J. Am. Chem. Soc. 78*, 4180.
Wechter, W. J., and Smith, D. C. (1968), *Biochemistry 7*, 4064.
Yano, M., and Hayatsu, H. (1970), *Biochim. Biophys. Acta 199*, 303.

Interactions between Cytidine and Its Cation in Polycytidylic Acid, Cytidylyl-3′-cytidine, and Cytidine Aggregates*

Thérèse Montenay-Garestier† and Claude Hélène

ABSTRACT: Fluorescence and phosphorescence titration curves of cytidine molecules dispersed in a water–propylene glycol glass (1:1, v/v) at 77°K have a classical sigmoidal shape. On the contrary, in cytidine aggregates formed in frozen aqueous solutions at 77°K, these titration curves exhibit an anomalous behavior. A luminescence intensity maximum is observed at a pH value close to the ground-state p*K*. The same phenomenon is observed at 77°K for poly C in ice and in a propylene glycol–water glass. Poly C exists in a double-stranded conformation at pH values close to the cytidine p*K*. The dinucleotide cytidylyl-3′:5′-cytidine dispersed as a single strand in this pH range behaves in the same way. At pH 4, the absorption spectrum of cytidine aggregates and poly C, deduced from low-temperature reflectance measurements, is shifted to longer wavelengths relative to that at pH 2 or 6. These observations are interpreted in terms of charge-transfer interactions between cytidine and its cation in stacked structures, where cytidine protonated on nitrogen 3 would behave as the acceptor and neutral cytidine as the electron donor.

A few homopolynucleotides, *e.g.*, poly A (Rich *et al.*, 1961; Adler *et al.*, 1969), and poly C (Fasman *et al.*, 1964; Hartman and Rich, 1965; Guschlbauer, 1967), form double-stranded structures in the acid pH range. Hydrogen bonding between bases and their cations as well as electrostatic forces are supposed to play a major role in maintaining these structures. However, other interactions such as those due to charge-transfer complex formation between a base and its cation could also help stabilize the double-stranded conformation.

Besides oligo- and polynucleotides, stacked bases can also be obtained in frozen aqueous solutions, the freezing of which induces the formation of microcrystalline aggregates (Wang, 1965; Bruice and Butler, 1965; Hélène *et al.*, 1968). These aggregates can be thought of as model structures for studies of electric interactions between nucleic acid bases (either neutral or ionized form) and have been used already to study excitation energy transfer (Hélène and Montenay-Garestier, 1968).

We have studied the pH dependence of the luminescence properties of the dinucleotide CpC, the polynucleotide poly C, and of cytidine aggregates in frozen solutions at 77°K. In all cases, a new fluorescence band appears for a pH value close to the ground-state cytidine p*K* that leads to anomalous luminescence titration curves. Reflectance spectra reveal the formation of molecular complexes between cytidine and its cation. The contribution of charge-transfer interactions to the stabilization of these complexes for both the ground state and the lowest excited singlet state is discussed.

Materials and Methods

Cytidine and cytidylyl-3′:5′-cytidine (CpC), were purchased from California Corp. for Biochemical Research. Poly C has been kindly supplied to us by Dr. M. Leng. Propylene glycol (B. D. H. Laboratory Reagent) has been distilled before use. The pH of the solutions was adjusted with microvolumes of hydrochloric acid or sodium hydroxide. All pH measurements were performed at room temperature with a Tacussel TS 40 N pH meter-titrimeter.

Spectroscopic studies were carried out with a Cary spectrophotometer Model 14. For low-temperature reflectance spectra, this apparatus was equipped with the total diffuse reflectance accessory adapted for low-temperature measurements as described elsewhere (T. Montenay-Garestier and C. Hélène, in preparation).

Thermal denaturation curves were obtained on a Cary Model 15 spectrophotometer.

Luminescence spectra at 77°K were recorded on a Jobin-Yvon spectrofluorimeter equipped with a xenon XBO 250-W lamp, two-quartz prism monochromators, and a 1P28

* From the Centre de Biophysique Moléculaire, La Source, 45-Orléans, France.
† Assistante au Muséum National d'Histoire Naturelle, Laboratoire de Biophysique 75-Paris-5ème, France.

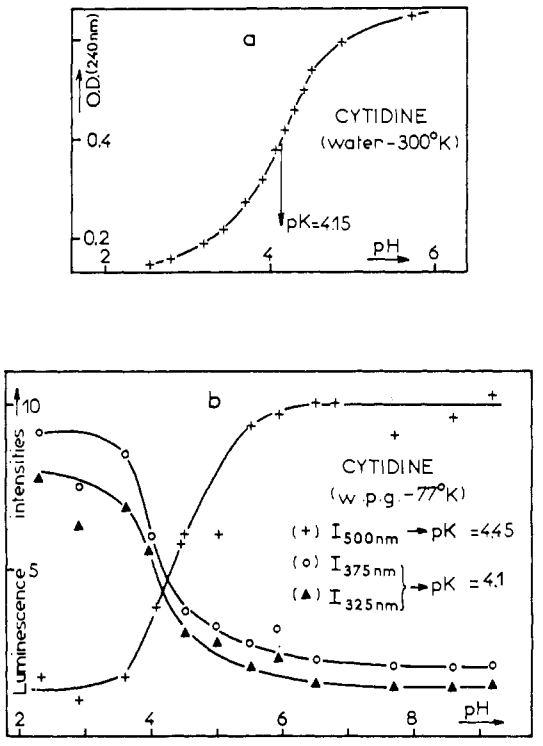


FIGURE 1: Cytidine studies. (a) Absorbance of a cytidine aqueous solution (10^{-4} M) measured at 240 nm *vs.* pH. (b) Luminescence titration curves of cytidine 10^{-3} M in water–propylene glycol mixture (1:1, v/v) at 77°K. pH dependence of fluorescence intensities in arbitrary unit at 325 nm (▲) and 375 nm (○) and phosphorescence intensity (+) at 500 nm in arbitrary unit. In this latter curve, the zero on the ordinate scale is translated by 0.5 unit.

photomultiplier. The sample was contained in a 2-mm i.d. quartz tube. The tube could be mechanically centered in a quartz dewar containing liquid nitrogen. The excitation wavelength was chosen at 280 nm. An M.T.O. filter (λ_{max} 278 nm, $\Delta\lambda_{1/2}$ = 17 nm) was placed in the excitation beam to prevent overlapping of scattered light and fluorescence. Emission spectra were corrected to take into account photomultiplier sensitivity and monochromator transmission.

Results

Spectrophotometric titration of cytidine in equeous solutions at room temperature enables us to determine the ground-state p*K* (Figure 1a). Experiments, performed at two different cytidine concentrations (10^{-4} or 10^{-2} M), give the same p*K* value (4.15) in excellent agreement with that determined by Fox and Shugar (1952).

The pH dependence of the luminescence (fluorescence and phosphorescence) of dispersed cytidine molecules is measured at 77°K on glasses formed from a frozen mixture of water–propylene glycol (1:1, v/v). The pH is determined in water at room temperature. Previous experiments on nucleotides, dinucleotides, and polynucleotides have shown that nucleosides and nucleotides from aggregates in frozen aqueous solutions (Hélène and Michelson, 1967; Hélène *et al.*, 1968). However, these aggregates are not obtained in water–propylene glycol at 77°K. If protonation equilibrium is fast as compared with the excited-state lifetime, the pH dependence of fluorescence and phosphorescence would allow the determination of the p*K*'s of the lowest excited singlet and triplet states, respectively (Weller, 1961; Wehry, 1967). This is very unlikely in rigid medium especially for the lowest singlet state whose lifetime is very short. In fact, the p*K*'s deduced from the fluorescence and phosphorescence titration curves of cytidine in water–propylene glycol at 77°K are 4.1 and 4.45, respectively (Figure 1b), and are very close to the ground-state p*K* determined at room temperature in aqueous solution (Figure 1a). The sigmoidal curves shown in Figure 1b are very similar to those reported by Longworth *et al.* (1966) with frozen solutions of adenine and its derivatives or guanosine in ethylene glycol–water mixture (1:1, v/v) or by Börresen (1963), with purine and pyrimidine in aqueous solutions.

The p*K* of the lowest excited singlet and triplet states, pK_S and pK_T, can be calculated from the shift in the fluorescence and phosphorescence O–O bands of the conjugate acid and base forms, and the ground-state p*K*, pK_G (Förster, 1950; Weller, 1961) (for a discussion on the different methods used to determine excited-state p*K*'s, see Wehry and Rogers, 1965).

$$pK_{S,T} - pK_G = (\nu_C - \nu_{CH^+})\frac{hc}{2.303kT}$$

where ν_C and ν_{CH^+} represent transition frequencies in wave numbers for cytidine, C, and its cation, CH^+, respectively. The difference, $\nu_C - \nu_{CH^+}$, is about 300 cm^{-1} both for the lowest excited singlet and triplet states. Thus p$K_{S,T}$ − pK_G is about 2.4 at 77°K and 0.7 at 273°K. Since the measurements which are described below are done in ice, equilibria are assumed to be "frozen in" at 273°K. Therefore, the p*K* of cytosine in the lowest excited singlet and triplet states are expected to be very close to the ground-state p*K*.

Frozen aqueous solutions of cytidine in acid medium (pH 3) exhibit fluorescence whose maximum is located at 340 nm, and also a weak phosphorescence. In the neutral form (pH 7), fluorescence predominates. Phosphorescence is located at 425 nm in acid medium and at 430 in neutral medium (Figure 2). Kleinwächter *et al.* (1966) have suggested that protonation on N-3 of the pyrimidine ring shifts the corresponding n–π^* level to higher energy. The probability of intersystem-crossing ($S_1 \rightsquigarrow T_1$) is thus decreased and the ratio of the fluorescence to phosphorescence quantum yield is enhanced. In the anionic molecules (pH >12.5), the opposite is observed: the quantum yield of phosphorescence is higher than that of fluorescence in agreement with the findings of Kleinwächter *et al.* (1966).

Luminescence studies of frozen aqueous solutions of cytidine (10^{-3} M) *vs.* pH show an anomalous behavior of this luminescence in the vicinity of the cytidine ground-state p*K* (Figure 3a). When pH decreases, a progressive shift of the total emission spectrum maximum toward longer wavelengths is observed (Figure 2). This shift is accompanied by a large increase of the phosphorescence intensity followed by a decrease. The curves of phosphorescence and fluorescence intensities *vs.* pH exhibit a peak at pH 4.1 (Figure 3a) whereas they are sigmoidal in the case of a water–propylene glycol mixture (Figure 1b). The half-width of the peak is about 0.8 pH unit (Figure 3a). At pH 4.1, the phos-

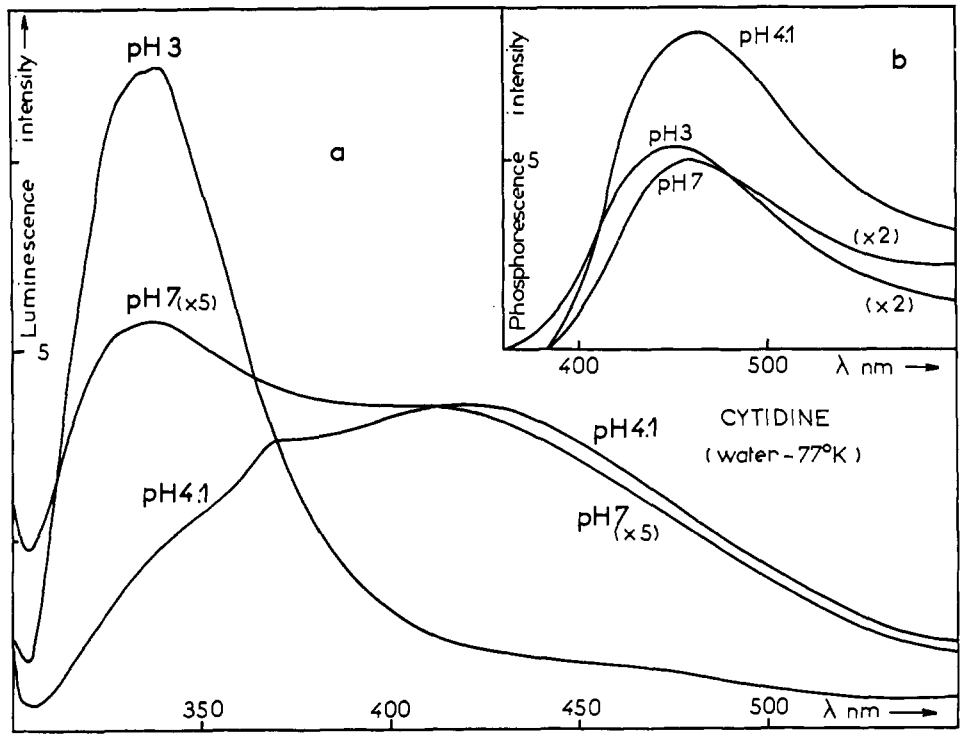


FIGURE 2: Luminescence and phosphorescence spectra. (a) Corrected luminescence spectra of frozen aqueous solutions at 77°K of cytidine (10^{-3} M) at pH 3.7 and 4.1. (b) Corrected phosphorescence spectra of the same solutions. The intensities are reported in arbitrary unit.

phorescence maximum is located at 460 nm and its intensity is about fivefold higher than that at pH 3 or 7. By subtracting the phosphorescence from the whole luminescence, the fluorescence can be obtained and is shown in Figure 4. A wide fluorescence band (100-nm half-width) extending as far as 500 nm is observed and its maximum is at 380 nm.

Similar experiments at constant ionic strength (0.1 M NaCl) give the same results (Figure 3b). Therefore sodium and chloride ions are not involved in the observed phenomenon. However, under these conditions, the peak maximum is obtained at pH 3.7 (instead of at 4.1 in the absence of NaCl). Aqueous cytidine solutions containing 5 or 33% ethanol show the same phenomenon (Figure 3c). The peak maximum is then obtained at pH 3.9 and 4, respectively. As outlined above, in frozen aqueous solutions cytidine molecules form aggregates. The "anomalous" luminescence titration curves obtained when cytidine molecules are not dispersed should be attributed to interactions between molecules included into these aggregates. The exact structure of aggregates is unknown but experimental results are consistent with a microcrystalline structure (Wang, 1965; Keller and Breen, 1965; Bruice and Butler, 1965; Hélène *et al.*, 1968). This implies both "vertical" stacking and "horizontal" hydrogen bonding. We have also shown (Montenay-Garestier and Hélène, 1968) that the ice host may induce the formation of molecular complexes of charge-transfer type between tryptophan and nucleosides. These complexes have well-characterized fluorescence and reflectance spectra. The anomalous behavior reported above in the case of cytidine aggregates could be attributed either to vertical stacking or to hydrogen-bond interactions between two molecules in the same horizontal plane. It should therefore be found in solutions of poly C which is double stranded in the acid pH range (Fasman *et al.*, 1964; Hartman and Rich, 1965; Guschlbauer, 1967). For poly C (10^{-4} M in monomer) in sodium cacodylate buffer (1.6×10^{-4} M)–NaCl (0.1 M) a peak similar to that obtained on cytidine aggregates is observed at pH 4.5, as shown in Figure 5b. Thermal denaturation, under these experimental conditions, is a cooperative process corresponding to the double-helix dissociation (Figure 5b). However vertical or horizontal interactions could explain the above luminescence results as well as in the case of cytidine aggregates.

To distinguish between these two possibilities, a luminescence titration of the dinucleotide CpC (10^{-3} M in cytosine) has been performed. The interactions between the stacked bases in this dinucleotide lead to the formation of an excimer (excited dimer) in water–propylene glycol at 77°K (Hélène and Michelson, 1967; Eisinger *et al.*, 1966). This dinucleotide does not form a double-stranded structure. The thermal denaturation of CpC (10^{-4} M in monomer) in water–propylene glycol mixture (1 M in sodium acetate) at pH 4.7 shows no cooperative process between -30 and $+90°$ (the freezing point of the solution under these conditions is $-31°$). Therefore, CpC in this temperature range does not form a double helix in this solvent. Vertical interactions between stacked bases are the only possible interactions. As shown, in Figure 5a an "anomalous" behavior of the luminescence titration is also observed as in the case of poly C or cytidine aggregates.

The characteristic shape of the luminescence titration curves of poly C, CpC, and cytidine aggregates at 77°K in the vicinity of the cytidine pK can thus be ascribed to interactions between stacked molecules. It was necessary to determine whether such interactions might also modify the absorption spectrum

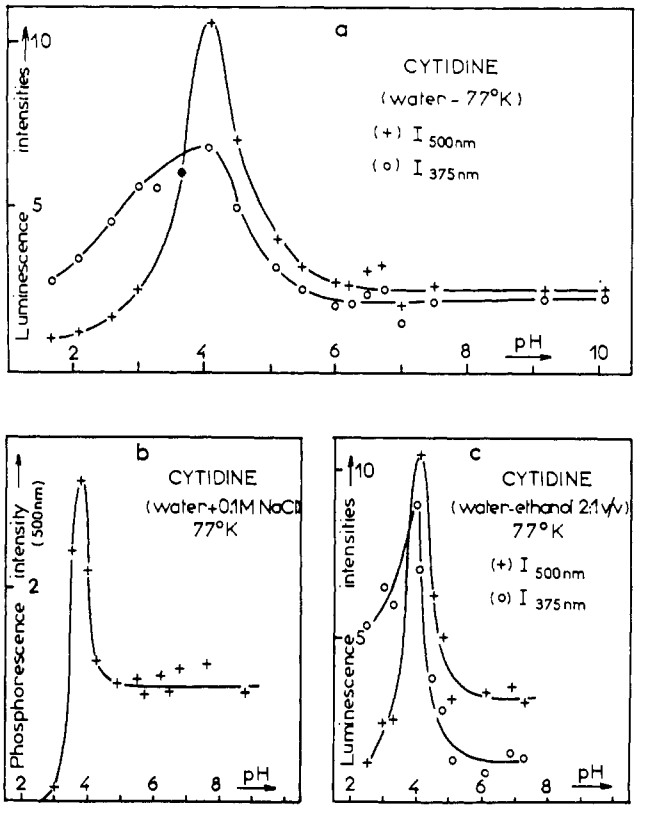


FIGURE 3: Luminescence titration curves of 10^{-3} M frozen cytidine solutions at 77°K in arbitrary unit *vs.* pH. (a) Intensity measured at 500 nm (+) and at 375 nm (O) in a frozen aqueous solution. (b) Intensity measured at 500 nm for an aqueous solution containing 0.1 M NaCl. (c) Intensity measured at 500 nm (+) and 375 nm (O) for an aqueous solution containing ethanol (33%).

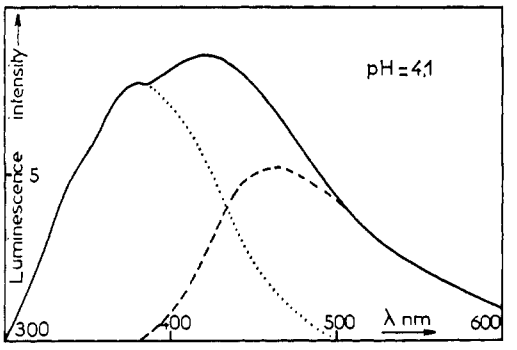
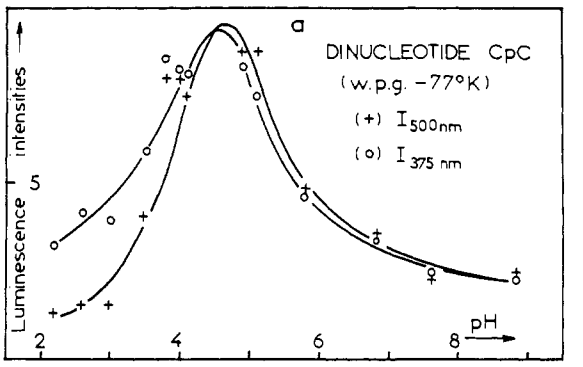


FIGURE 4: Emission spectrum of a 10^{-3} M frozen aqueous cytidine solution at 77°K at pH 4.1. The fluorescence band (. . .) is deduced from the total luminescence spectrum (——) by subtracting the phosphorescence spectrum (– – –). Intensities are in arbitrary unit.



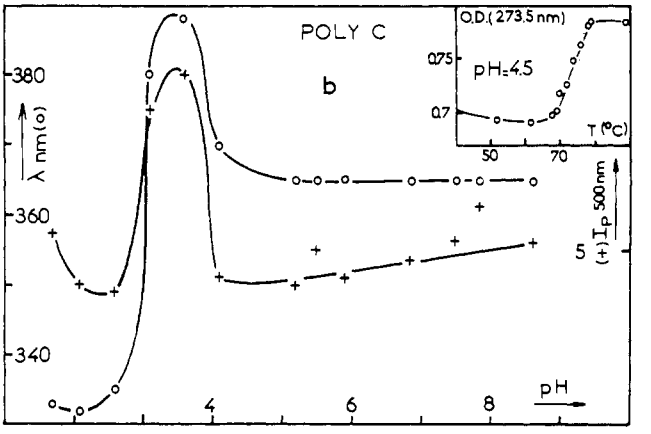


FIGURE 5: Luminescence titration curves (a) of CpC (10^{-3} M in monomer) in water–propylene glycol mixture at 77°K; the intensities measured at 500 nm (+) and 375 nm (O) are plotted in arbitrary unit *vs.* pH; (b) of poly C (5×10^{-5} M in monomer) in 0.1 M NaCl and 1.6×10^{-4} M sodium cacodylate buffer at 77°K. (+) Intensity at 500 nm in arbitrary unit. (O) pH dependence of the wavelength of the fluorescence band maximum. Insert: thermal denaturation curve of the same solution at pH 4.5 (the T_m is about 73°).

of these compounds. In fluid solutions, we have not been able to detect the effect of these interactions on the absorption spectrum of cytidine and CpC. The opacity of the frozen samples precludes the use of standard absorption spectrophotometry. Absorption spectra were deduced from reflectance spectra obtained with frozen samples at −20° (Montenay-Garestier and Hélène, 1968). Figure 6a shows the spectra obtained with cytidine 5×10^{-3} M, at pH 6.3, 4.2, and 2.0. The spectra obtained with cytidine at pH 6.3 and 2.0 are characteristic of cytidine and its cation, respectively. In fluid solution there is a continuous change from one spectrum to the other when pH decreases from 6.3 to 2.0 with well-defined isosbestic points. This is not the case in frozen aqueous solutions. The absorption spectrum at pH 4.2 extends to much longer wavelengths than those of cytidine and its cation (Figure 6a). This new absorption at long wavelengths can also be seen in the reflectance spectrum of poly C at pH <5 where a double-stranded conformation is formed (Figure 6b). Hypochromism is also observed at wavelengths longer than 300 nm in aqueous solutions when double-stranded poly C undergoes thermal denaturation (Guschlbauer, 1967), even at low pH values where no loss of protons takes place (loss of proton by cytidine would be accompanied by an hypochromism at 300 nm).

This strongly suggests that the same molecular interactions are responsible both for the anomalous behavior of the luminescence titration curves and the new absorption spectrum.

Discussion and Conclusion

The peak in both fluorescence and phosphorescence titration curves is observed at a pH value very close to the cytidine

pK which is practically the same in either the ground or the lowest excited singlet and triplet states at 77°K. It is therefore very likely that this peak is due to interactions between protonated and neutral cytidine molecules which are in equal quantities when pH = pK. Such interactions could lead to the formation of a charge-transfer complex in the ground state or an excimer[1] in which charge-transfer interactions are thought to be important (McGlynn *et al.*, 1965). With this hypothesis, we can calculate the luminescence titration curves in a semiempirical way, for example, in the case of phosphorescence of 10^{-3} M cytidine.

Let c_0 be the total cytidine concentration, ρ_c the phosphorescence quantum yield of neutral cytidine at the excitation wavelength, ρ_x that of protonated cytidine (concentration X), and ρ that of the complex. The pK value was taken as 4.1. This value corresponds to the maximum on the experimental curve (Figure 3a). In fluid solution, the equilibrium between protonated and neutral cytidine molecules can be written as

$$CH^+ \overset{K}{\rightleftharpoons} C + H^+$$

$$K = \frac{[C][H^+]}{[CH^+]}$$

We suppose that the freezing of aqueous solutions keeps the equilibrium concentrations defined above. The whole phosphorescence intensity is the sum of the intensities emitted by the complex (concentration X) and by neutral cytidine molecules for pH $\geqslant$ pK (concentration $c_0 - 2X$) or protonated molecules if pH $\leqslant$ pK. It is assumed that for pH $\geqslant$ pK, each CH^+ cation forms a complex with a neutral molecule and for pH $\leqslant$ pK each C molecule forms a complex with a CH^+ cation.

The following equations can be written

(1) pH $\geqslant$ pK

$$I_p = \rho_c(c_0 - 2X) + \rho X$$

$$I_p = \rho_c c_0 + \frac{c_0[H^+]}{K + [H^+]}(\rho - 2\rho_c)$$

(2) pH $<$ pK

$$I_p = \rho_x(c_0 - 2X) + \rho X$$

$$I_p = \rho_x c_0 + \frac{c_0 K}{[H^+] + K}(\rho - 2\rho_x)$$

The experimental values of the phosphorescence intensity at pH 2, 4.1 and 7.5 were used to determine the relative values of ρ_x, ρ, and ρ_c, respectively. As shown in Figure 7, the experimental and calculated curves are in quite good agreement. Therefore complex formation between cytidine and its cation can explain the observed luminescence titration curves.

[1] An excimer is a complex formed between one molecule in its lowest excited state, and a neighboring one in its ground state. The word exciplex is used when the two molecules are different chemical species (Birks, 1967).

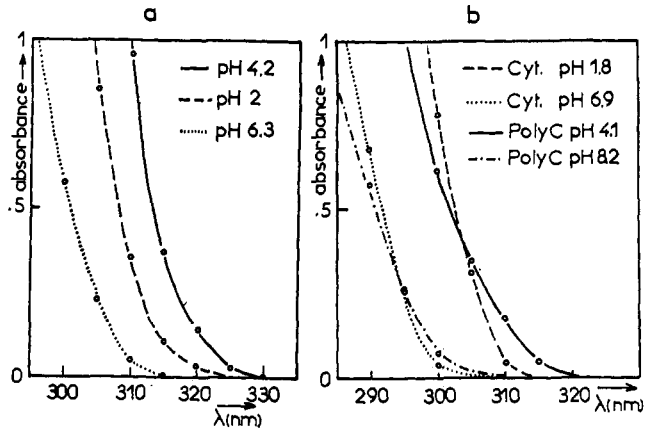


FIGURE 6: Reflectance spectra. (a) Of cytidine (5×10^{-3} M) in aggregates at pH 4.2 (——), pH 2 (– – –), and pH 6.3 (. . .); (b) of cytidine (10^{-3} M) and poly C (10^{-3} M in monomer) in frozen aqueous solutions containing 0.05 M NaCl at different pH values.

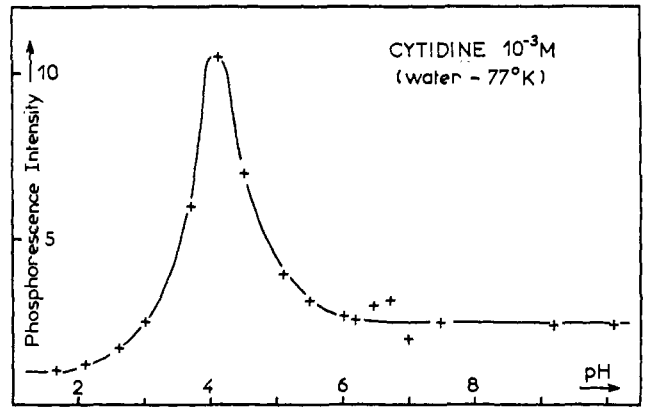


FIGURE 7: Calculated curve and experimental points (+) corresponding to the pH dependence of the phosphorescence intensity of a 10^{-3} M frozen aqueous solution of cytidine measured at 500 nm (arbitrary unit) (*cf.* Figure 3a). The following equations were used to calculate the semiempirical curve (see text): pH $\geqslant$ pK, $I_p = 2.5 + (16\,[H^+]/K + [H^+])$; pH $\leqslant$ pK, $I_p = 1 + (19K/K + [H^+])$.

For pH values close to the pK, the absorption spectrum of cytidine aggregates extends to wavelengths longer than that of neutral and protonated cytidine. Thus interactions between cytidine and its cation in the complex do occur in the ground state. The stabilization of the complex is very likely due to both Van der Waals–London forces and charge-transfer interactions. The latter could be responsible for the new absorption observed in frozen aqueous solutions of cytidine at pH 4.2. At this pH, the fluorescence spectrum is markedly shifted to longer wavelengths and broadened as compared with that of cytidine or its cation as expected for an electron donor–acceptor complex. The shift of the fluorescence spectrum is much more important than that of the absorption spectrum. This could indicate that charge-transfer interactions are more important in the lowest excited singlet state than in the ground state This is often observed for weak electron donor–acceptor complexes (Mataga and Murata, 1969). In the complex, neutral cytidine molecules would behave as electron-donor and protonated cytidine molecules as electron

acceptor. A similar complex between acridine and its cation has been already observed by Hammond (1964).

Acknowledgments

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References

Adler, A. J., Grossman, L., Fasman, G. D. (1969), *Biochemistry 8*, 3846.
Birks, J. B. (1967), *Nature 214*, 1187.
Börresen, H. C. (1963), *Acta Chem. Scand. 17*, 921.
Bruice, T. C., and Butler, A. R. (1965), *Fed. Proc. 24*, S-45.
Eisinger, J., Gueron, M., Shulman, R., and Yamane, T. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 1015.
Fasman, G. D., Lindblow, C., and Grossman, L. (1964), *Biochemistry 8*, 1015.
Förster, T. (1950), *Z. Elektrochem. 54*, 42.
Fox, J. J., and Shugar, D. (1952), *Biochim. Biophys. Acta 9*, 369.
Guschlbauer, W. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 1441.
Hammond, P. R. (1964), *Nature 201*, 922.
Hartman, K. A., and Rich, A. (1965), *J. Am. Chem. Soc. 87*, 2033.
Hélène, C., and Michelson, A. M. (1967), *Biochim. Biophys. Acta 142*, 12.
Hélène, C., and Montenay-Garestier, T. (1968), *Chem. Phys. Let. 2*, 25.
Hélène, C., Ptak, M., and Santus, R. (1968), *J. Chim. Phys. 65*, 160.
Keller, R. A., and Breen, D. E. (1965), *J. Chem. Phys. 43*, 2562.
Kleinwächter, V., Drobnik, J., Augenstein, L. (1966), *Photochem. Photobiol. 5*, 579.
Longworth, J. W., Rahn, R. O., and Shulman, R. G. (1966), *J. Chem. Phys. 45*, 2930.
Mataga, N., and Murata, Y. (1969), *J. Am. Chem. Soc. 91*, 3144.
McGlynn, S. P., Armstrong, A. T., and Azumi, T. (1965), *in* Modern Quantum Chemistry, Vol. 3, Sinanoglu, O., Ed., New York, N. Y., Academic, p 203.
Montenay-Garestier, T., and Hélène, C. (1968), *Nature 217*, 844.
Rich, A., Davies, D. R., Crick, F. H. C., Watson, J. D. (1961), *J. Mol. Biol. 3*, 71.
Wang, S. Y. (1965), *Fed. Proc. 24*, S-71.
Wehry, E. L. (1967), *in* Fluorescence, Guilbault, G. G., Ed., New York, N. Y., Marcel Dekker, p 37.
Wehry, E. L., and Rogers, L. B. (1965), *Spectrochim. Acta 21*, 1976.
Weller, A. (1961), Progress in Reaction Kinetics, Vol. 1, London, Pergamon, p 187.

Metabolism of Ubiquinone-7*

Isuke Imada, Masazumi Watanabe, Norichika Matsumoto, and Hiroshi Morimoto

ABSTRACT: Several new metabolites of ubiquinone-7 and one of their conjugates were obtained from the excrements and the tissues of rats and rabbits to which ubiquinone-7 had been administered. The three metabolites and one conjugate obtained from the excrements were identified as 2,3-dimethoxy-5-methyl-6-(3′-methyl-4′-oxopentyl)-1,4-benzoquinone (IV), *d*-2,3-dimethoxy-5-methyl-6-(3′-carboxy-3′-methylpropyl)-1,4-benzoquinone (V), *trans*-2,3-dimethoxy--5-methyl-6-(5′-carboxy-3′-methyl-2′-pentenyl)-1,4-benzoquinone (VII), and the disulfate XIII of the hydroquinone form of V, respectively, by comparison of their spectral data with those of synthetic samples. The earlier assumption that 2,3-dimethoxy-5-methyl-6-(5′-carboxy-3′-hydroxy-3′-methylpentyl)-1,4-benzoquinone lactone (III) is a metabolic end product was corrected and the lactone was proved to be an artifact formed from the conjugate of VII during the hydrolysis step.

The metabolite XIV from the adrenals was found to be an *ω-cis*-carboxylic acid which was formed by oxidation of the *cis*-methyl group in the terminal isoprene unit, while the metabolite XVIII from the ovaries was found to be the 26,27-dihydro compound of XIV, and they were obtained as the cholesteryl esters. The metabolic pathway of ubiquinone is discussed.

On the assumption that ubiquinone is excreted in the urine as a conjugate of 2,3-dimethoxy-5-methyl-6-(5′-carboxy-3′-hydroxy-3′-methylpentyl)-1,4-benzoquinone lactone (III), presumably as a glucuronide similarly to the excretion of α-tocopherol (Simon *et al.*, 1956a,b), Gloor *et al.* (1966) and Wiss and Gloor (1966) investigated III in the urine of rats to which labeled Q-9[1] had been administered, by the isotope dilution method and confirmed the presence of III in the urine. The authors investigated the metabolites of Q-7 (I), using the facts that a metabolite retaining the partial

* From the Chemical Research Laboratories, Takeda Chemical Industries, Ltd., Juso, Osaka, Japan. *Received January 20, 1970.* Ubiquinones and Related Compounds. XVII. (On the Components of Yeast. XIX).

[1] Abbreviation used is: Q-*n*, ubiquinone-*n*.