TRIPLET-TRIPLET ENERGY TRANSFER BETWEEN NUCLEIC ACIDS DERIVATIVES IN FROZEN AQUEOUS SOLUTIONS.

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We have previously shown by emission spectroscopy that energy transfer may occur in dinucleotides (HELENE, DOUZOU and MICHELSON 1965,1966). We wish to report results on the phosphorescence at 77°K of frozen aqueous mixtures of nucleosides or derivatives which show that :

I) aggregates are formed in the freezing of aqueous solutions,

2) triplet-triplet energy transfer may occur in mixed aggregates but proton transfer is not implied as shown by studies of methylated derivatives,

3) hydrogen bond formation between two derivatives leads to emission quenching.

These results strongly suggest that the U.V.-induced triplet state in DNA (RAHN, SHULMAN and LONGWORTH, 1965) is that of thymine reached by triplet-triplet energy transfer.

MATERIALS AND METHODS.

The nucleosides were purchased from either SIGMA or CALBIOCHEM. 3-methylthymidine (MT) and I-methyl-N_4-acetyl-cytosine (AcC) were synthesized by Drs A.M. MICHELSON and F.POCHON who are gratefully acknowledged. I,3 dimethyluracil (DMU) and I,3 dimethylthymine (DMT) were prepared by action of dimethylsulfate on uracil and thymine respectively, according to DAVIDSON and BAUDISH (1926). They were recrystallized from acetone + petroleum ether. Emission measurements were carried out with an AMINCO-KEIRS spectrophosphorimeter. Phosphorescence decay-times were measured with a TEKTRONIX oscilloscope equipped with a polaroïd camera.

RESULTS.

I°) Aggregate formation in frozen aqueous solutions at 77°K.

In the freezing of an aqueous solution , it has been supposed that solute molecules are excluded from the ice crystal and form aggregates.

This has been proposed to explain some ionic reactions (BRUICE and BUTLER 1965), dipolar broadening of electron-spin resonance spectra (ROSS 1965) as well as photodimerization (WANG 1961, 1965) in frozen aqueous solutions. Molecular interactions generally quench the phosphorescence in aggregates (STERNLICHT, NIEMAN and ROBINSON 1963). In agreement with this, the phosphorescence of frozen aqueous solutions of purine, adenosine and guanosine is very weak compared to nonaqueous solutions (at the same concentration). Aggregates may be destroyed by addition of salts (BRUICE and BUTLER 1965) or organic solvents like ethanol or glycerol (WANG 1965) before freezing of aqueous solutions.

Addition of 1% ethanol to an aqueous solution of purine before cooling leads to a twenty-fold increase of the phosphorescence intensity at 77°K, compared to the pure aqueous solution. At the same time, a blue-shift of both excitation and phosphorescence spectra is observed whereas the triplet-state lifetime slightly increases (from I,6s to I,8s). Further addition of ethanol (up to 75%) only leads to very slight modifications of the phosphorescence characteristics.

Similar results are obtained with frozen aqueous solutions of purine nucleosides although the phosphorescence enhancement by ethanol is lower than with purin (three times and four times for adenosine and guanosine respectively). Addition of NaCl (0,5M) to aqueous solutions before freezing leads to similar results. The phosphorescence enhancement is higher than with ethanol (six and ten times for adenosine and guanosine, respectively).

All these results suggest that aggregates are formed in the freezing of aqueous solutions. When ethanol or NaCl is added, it is likely that ethanol molecules or solvated cations would be excluded from the water crystal together with solute molecules and lead to destruction of aggregates and hydrogen-bond formation. The consequences would be both a phosphorescence enhancement and spectral shifts as has been indeed observed. Hydrogen-bond formation has been already proposed to explain the enhancement of benzophenone phosphorescence in hydrocarbon glass at 77°K by addition of ethanol (RICHTOL and KLAPPMEIER 1964).

2°) Energy-transfer in frozen aqueous solutions of nucleic acids derivatives

When an equimolecular aqueous mixture of adenosine and thymidine is cooled down to 77°K, the phosphorescence characteristics (λ_{max} = 470 mμ ; τ = 0,21s) are completely different from those of adenosine (λ_{max} = 435mμ ; τ = 2,3s). The phosphorescence of the equimolecular mixtures A + T, G + T, AcC + T, C + T are nearly identical (table I). These results clearly show that thymidine is the phosphorescent site. A very weak phosphorescence (λ_{max} = 470mμ) may be detected in concentrated (10^{-2} M) aqueous solutions of thymidine at 77°K. An enhancement of the phosphorescence intensity and a shift of the phosphores-

TABLE I : Characteristic data (lifetime τ and λ max.) of the phosphorescence at 77°K of nucleic acids derivatifes and of frozen aqueous mixtures.

	τ (s)	λmax. (mμ)		τ (s)	λmax. (mμ)
Adenosine (A) (H_2O)	2,3	435	A + T (1:1) (5.10^{-3} M)	0,21	470
Guanosine (G) (H_2O)	1,2	430	G + T (1:1) (10^{-3} M)	0,22	470
Cytidine (C) (H_2O) (1)	0,6	435	C + T (1:1) (5.10^{-3} M)	0,22	470
1-methyl-N_4-acetyl cytosine (AcC) (H_2O)	0,32	425	AcC+T (1:1) (5.10^{-4} M)	0,21	475
Uridine (U) (A.H.) (2)	0,5	415	A + MT (1:1) (5.10^{-3} M)	0,26	465
Thymidine (T) (A.H.) (2)	0,4	445	AcC+MT (1:1) (5.10^{-4} M)	0,24	465
3-methylthymidine (MT) (A.H.) (2)	0,6	450	A +DMT (1:1) (5.10^{-3} M)	0,3	470
1,3-dimethylthymine (DMT) (A.H. or water + 10% ethanol)	0,5	455	AcC+DMT (1:1) (5.10^{-4} M)	0,3	465

(1) the phosphorescence of cytidine may be obtained with concentrated aqueous solutions (5.10^{-3} M).

(2) A.H. is ammonium hydroxide 1N.

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cence spectrum are observed in alkaline solutions (λmax = 445 mμ ; τ = 0,4s; at pH ≃ 11,6 measured at room temperature). In these conditions, thymidine has lost its N_3 proton (pK = 9,6). However, 3-methylthymidine (MT) and 1,3-dimethylthymine (DMT) which cannot loose a proton have a similar behaviour. Moreover, the very weak phosphorescence of the thymine derivatives (T,MT and DMT) in frozen aqueous solutions is also enhanced by addition of either 10% ethanol or 0,5 M NaCl (table 1).On the other hand, the absorption maximum of T is not modified by loosing the N_3 proton. Only a decrease of the molar extinction coefficient (i.e. the singlet-singlet transition probability) is observed. Thus, the loss of the N_3 proton may increase the singlet-triplet intersystem crossing probability without modification of the triplet-state characteristics.

In every one of the following equimolecular mixtures : A + T, A + MT, A + DMT, AcC + T, AcC + MT, AcC + DMT, the phosphorescence is similar to that of the thymine derivative (table 1). These results clearly show that neither hydrogen bonding nor proton transfer are a prerequisite for the observation of the thymine triplet-state.

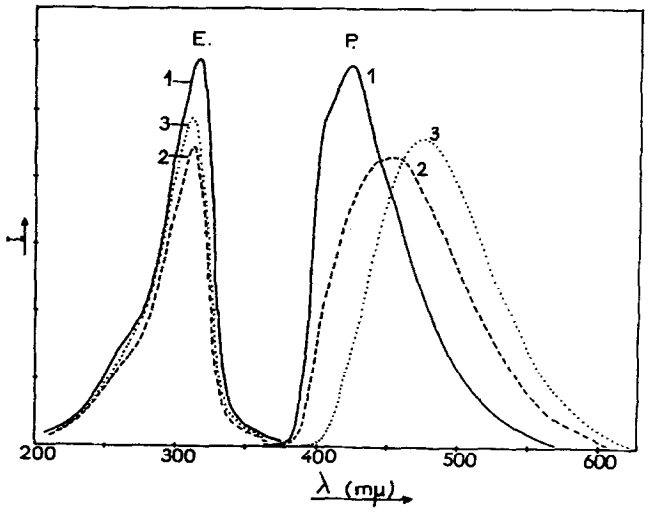

Figure I : Uncorrected excitation (E) and phosphorescence (P) spectra of AcC (1) and mixtures of AcC and T in the concentration ratios 10:1 (2) and 1:1 (3). The concentration of AcC is the same (5.10^{-4}M) in the three cases. The relative intensity (I) units are in the ratios 10 : 2,5 : 1 for 1 : 2 : 3 respectively. Frozen aqueous solutions at 77 K .

Moreover the excitation spectra of AcC and mixtures of AcC with the thymine derivatives are practically identical (figure 1). AcC may be selectively excited at 310 mμ, at which wavelength the thymine derivatives do not absorb light. Therefore, triplet-triplet energy transfer can only explain the appearance of the thymine phosphorescence.

No energy transfer is to be detected in the mixture of 5-bromocytidine (BrC) and thymidine (T) because the triplet state of BrC has an energy lower than that of T (HELENE et al. 1965). The behaviour of uracil derivatives may be compared to that of the thymine derivatives. The phosphorescence of G, AcC, A are quenched by addition of either uridine (U) or 1,3-dimethyluracil (DMU). Singlet-singlet energy transfer from G or AcC to U is ruled out by the fact tha the energy of the first excited singlet state of G or AcC is lower than that of U. Thus triplet-triplet energy transfer must occur.The triplet-state of the uracil derivatives may be detected in water + 10% ethanol or in alkaline solutions (as for the thymine derivatives) (table I).

The energy of the lowest triplet-state of T is lower than that of the lowest triplet-state of U. That difference as well as the great spin-spin interaction measured by electron-spin resonance (RAHN et al. 1965) (HELENE and SANTU

unpublished results) strongly suggest that the triplet is localized in the neighbourhood of the 5-6 double bond.

3°) Hydrogen-bonded complexes between two derivatives in frozen aqueous solutions.

The phosphorescence and the fluorescence of AcC ($5\text{-}10^{-4}$ M) at 77°K are rapidly quenched by small amounts of guanosine. No sensitized phosphorescence of G may be observed. Since the first excited singlet state of AcC has an energy lower than that of G, singlet-singlet energy transfer from AcC to G is ruled out. The quenching may be explained by the formation of hydrogen-bonded pairs.

The high quenching yield of AcC luminescence by G, when the concentration ratio G : AcC is less than 0.1, is probably the result of energy delocalization in the aggregates in which the hydrogen-bonded pairs act as energy-sinks. BrC and G seem to form hydrogen-bonded pairs too. Similar results have been already obtained with mixtures of carbazole and acridine (EL-BAYOUMI and KASHA, 1961) and the emission quenching of carbazole explained by exciton interaction in hydrogen-bonded complexes.

DISCUSSION.

The triplet-state energy of nucleic acids derivatives in frozen aqueous solutions (aggregates) may be deduced from the phosphorescence spectra and triplet-triplet energy transfer studies. It decreases in the order :

$$G,\ AcC\ ,\ G > A > U > T > BrC$$

When aggregates are destroyed and hydrogen-bonds formed by addition of ethanol, the phosphorescence spectra shift towards shorter wavelengths. The smaller shift is that of cytidine. In these conditions, the triplet-state energy decreases as follows :

$$AcC > G > A > C,\ U > T > BrC$$

Among the 4 nucleotides of DNA, T has the lowest triplet-state energy whatever the conditions. The triplet-state of thymidine may be populated enough by triplet-triplet energy transfer as to record its phosphorescence. It is quite possible that the U.V.-induced triplet-state of both poly dAT and DNA would be that of thymidine reached by such a transfer rather than that of ionized thymidine as suggested by RAHN et al. (1965). Furthermore the influence of paramagnetic metal ions on both photodimerization of thymine (BERENDS, 1961) and U.V. induced radicals at 77°K in thymine and DNA (PERSHAN et al. 1964) suggest that these two photochemical processes occur via the triplet-state of thymine. It has been shown that the thymine triplet-state may be reached by triplet-triplet energy-transfer. It is likely, therefore, that thymine should be the preferred

site of U.V.-induced damages in D.N.A. . Work is now in progress to test the above hypothesis.

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