EXCITED STATE INTERACTIONS BETWEEN GUANINE AND CYTOSINE

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Summary

Fluorescence and phosphorescence from the dinucleotide guanylyl (3′ → 5′)cytidine (GpC) were studied in two low temperature solvent glasses buffered to pH 6. The dinucleotide exhibits hypochromism in 1:1 ethylene glycol:water glass but not in 91:9 ethanol:water glass indicating that the bases are sufficiently close in the former matrix to allow an exciton interaction. Comparison of luminescence spectra with those of the mononucleotides indicates that the singlet state of an exciplex is formed in 1:1 ethylene glycol:water where the bases are close together, but much less of this exciplex is formed in 91:9 ethanol:water where the bases are not as close. In both solvent systems phosphorescence similar to the emission from the triplet state of guanosine monophosphate is observed. The phosphorescence yield of GpC is lower in 91:9 ethanol:water glass than in 1:1 ethylene glycol: water glass and in ethanol:water; exciplex phosphorescence is also observed on the tail of the main phosphorescence band. The spectral region in which the exciplex emission occurs is shown to be consistent with it being due to a charge transfer interaction between the guanyl and cytidyl moieties of GpC.

1. Introduction

Nucleic acid bases and dinucleotides luminesce in low temperature glasses and such systems have been widely used to examine the excited state properties of these molecules [1, 2]. There has been considerable interest in the excited state interactions between guanine and cytosine [3 - 5]. In this work attention has been focused on the fluorescence and phosphorescence which can be detected from the dinucleotide guanylyl(3′ → 5′)cytidine (GpC) in low temperature glasses [4 - 6].

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The bases in dinucleotides "stack" to different degrees in different solvents [3, 7, 8], *i.e.* the bases pivot around their common phosphate link and the interbase separation depends on the solvent. As a result the bases will be subject to different excited state interactions. In this paper the luminescence spectra of "isolated" guanine monophosphate (GMP) and cytosine monophosphate (CMP) molecules are compared with those of GpC molecules in two glassy solvents at pH 6 where the bases are stacked to different degrees.

2. Experimental procedure

GMP, CMP and GpC were obtained from the Sigma Chemical Co. The water was double distilled from alkaline potassium permanganate and the ethylene glycol was BDH reagent grade which had a transmission of 60% at 210 nm. 95% ethanol was refluxed with sodium hydroxide followed by fractional distillation. It gave a transmission of more than 50% at 210 nm.

The concentrations of the solutions used were 2×10^{-4} M for GMP and CMP and 1×10^{-4} M for GpC. Experiments performed on tenfold-diluted solutions gave the same results so there does not appear to be any self-association of mononucleotides or GpC occurring in the solvent glasses employed in this work. All solutions were buffered at pH 6 with 0.1 M sodium acetate. The two solvent systems used were 1:1 ethylene glycol:water (GW) and 91:9 ethanol:water (EW). These both form glasses at 77 K.

The solutions were contained in Suprasil quartz tubes and were rapidly cooled to 77 K. The phosphorescence measurements were performed using a phosphorimeter which employed front surface illumination at 265 nm. The detection system was calibrated using a quantum counter [9]. Scattered light from the excitation monochromator caused problems when this instrument was used to measure fluorescence from GpC solutions. All fluorescence spectra were therefore determined using another apparatus. A pulse of light at 264 nm from a JK Lasers *Q*-switched quadrupled neodymium laser irradiated the sample at right angles to the detection system. The fluorescence intensity was measured at the peak of the laser flash. The contribution of phosphorescence to this intensity was negligible at all wavelengths.

We have corrected the fluorescence spectra reported here using two spectral standards, 2-aminopyridine [9] and phenol [10], for the UV region. It should be noted that the ordinate (intensity) of the fluorescence and phosphorescence spectra are given in relative units of quanta per second per nanometre. The use of other units will result in shifts of the emission maximum [11].

Absorption spectra were measured at 77 K using a Hitachi EPS-3T absorption spectrometer with a rectangular cell 1 mm thick placed inside an unsilvered quartz Dewar. The spectrum of the solvent was also measured using the same apparatus and subtracted from the sample spectrum. The average bandwidth used was approximately 3 nm.

3. Results and discussion

The fluorescence and phosphorescence spectra of GpC in GW and EW are shown in Figs. 1 and 2 respectively.

3.1. Singlet states

The fluorescence spectra and yields of GMP, CMP and equimolar GMP plus CMP in EW are the same as those obtained in GW. However, as Fig. 1 shows, the fluorescence spectra of GpC in the two solvents are substantially

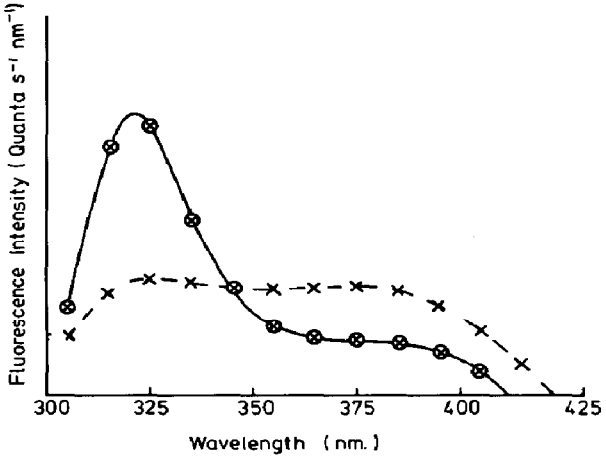

Fig. 1. Fluorescence spectra of GpC at 77 K in GW (— — —) and EW (——).

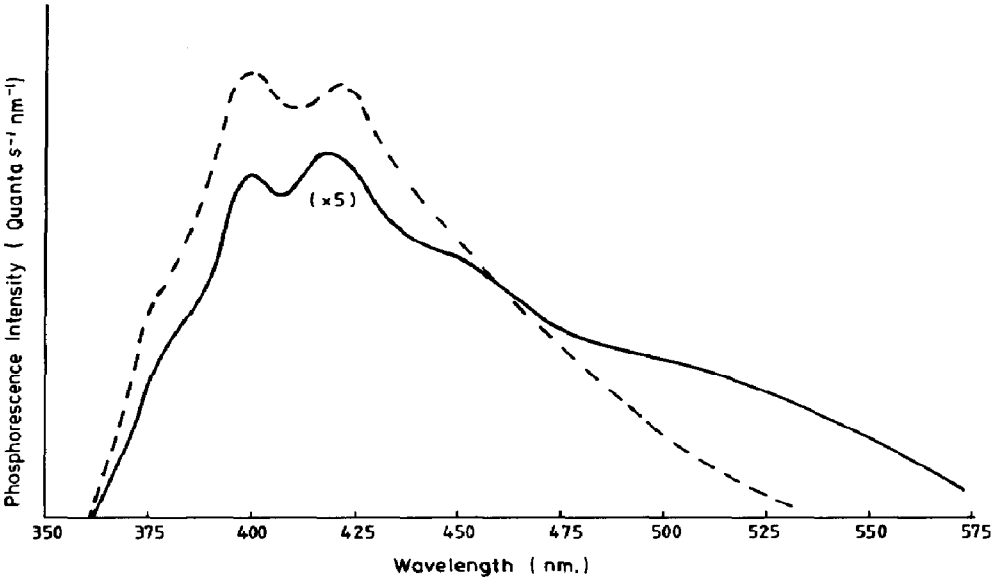

Fig. 2. Phosphorescence spectra of GpC at 77 K in GW (— — —) and EW (——).

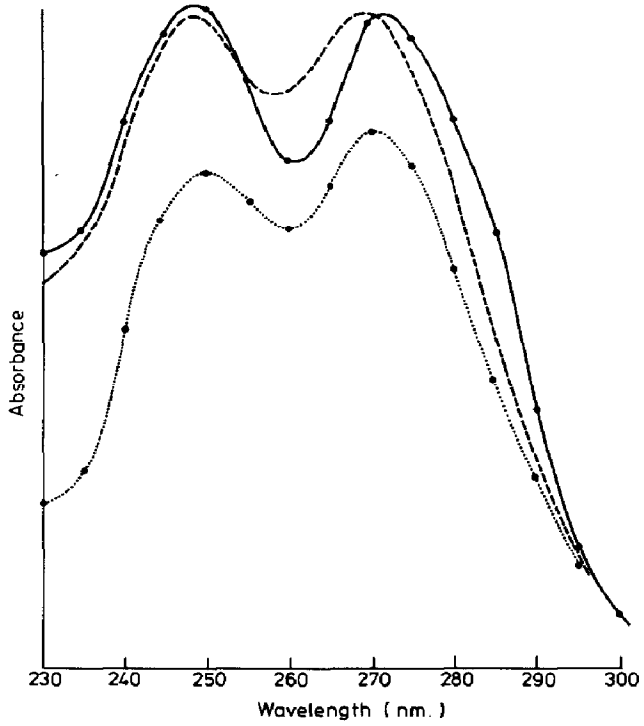

Fig. 3. Absorption spectra of GpC at 293 K (——, in GW and EW) and at 77 K (···, in GW; — — —, in EW).

different. In GW both exciplex and monomer emissions are observed with about equal intensities in agreement with previous work [4]. By contrast, in EW the exciplex emission is very much smaller than the monomer emission.

Two types of interaction can lead to the formation of exciplexes [12]. Exciton interaction arises from coupling of the transition dipoles of the exciplex components and the interaction energy depends on r^{-3} where r is the intermolecular distance. The strong coupling exciton type of interaction only occurs at small intermolecular separations. The second type of interaction is due to charge transfer. In this case the interaction energy depends on r^{-2} and therefore it is a longer-range interaction than the exciton interaction. Since the formation of exciplexes is very dependent on intermolecular separation, the difference in the amount of exciplex emission observed in this work is probably related to different configurations of the bases in the two solvents used. This is confirmed by the absorption spectrum of GpC where hypochromism is observed in GW at 77 K after allowing for a 10% contraction of the solvent but not in EW (see Fig. 3) [13, 14].

The fluorescence spectrum for GpC in EW is similar to that obtained for "isolated" CMP. However, because of the similarity of the fluorescence spectra for CMP and GMP, assignment of the fluorescence from GpC to the cytosine moiety must be made with caution.

It is interesting to note that Morgan and Callis [15] observed exciplex fluorescence from GpC in a 70:30 mixture of ethylene glycol:water at 77 K. However, they found that cytidylyl(3′ → 5′)guanosine (CpG) showed only monomer emission which they believe to arise from the cytosine moiety. The degree of base stacking depends on the "order" of the bases (the nature of the linkage of each base to the phosphate group). Hypochromism and optical rotary dispersion measurements indicate that CpG is less well stacked than GpC in water at room temperature [16, 17]. The spectra obtained by Morgan and Callis for GpC and CpG are similar to those reported here for GpC in solvents which subject the bases to different degrees of stacking, and it seems likely that their results can also be explained in terms of different degrees of base stacking.

3.2. Triplet states

The phosphorescence observed from GpC in GW is the same as that observed from the triplet state of GMP in GW buffered with sodium acetate [4]. The phosphorescence spectrum observed for GpC in EW is similar in shape but has an additional weak emission band on the red tail of the GMP phosphorescence. The intensity of the main band is about five times greater in GW than in EW and therefore it is possible that the weak emission band with a maximum at about 500 nm may also be present in GW but may be masked by the emission from the main band in this solvent. Absorption and excitation spectra show no indication of an impurity being responsible for the additional weak emission observed in EW. The wavelength region where the weak emission on the red tail of the GMP phosphorescence appears (from about 475 to 575 nm) suggests that it may be associated with a state arising from a charge transfer interaction between the guanyl and cytidyl moieties of GpC.

Emission from the triplet states of charge transfer exciplexes in low temperature glasses has been shown [18] to have a maximum given by

$$\nu_{max} = E_{D|D^+} - R_{A^-|A} - C(r)$$

where E_{D/D^+} is the oxidation potential of the donor. In this system E_{GMP} = 0.95 V [19]. $R_{A^-|A}$ is the reduction potential of the acceptor. In this system R_{CMP} = −1.68 V [20]. $C(r)$ is a coulombic interaction term which is dependent on the inverse square of interbase separation and which can also vary as a result of the charge transfer state coupling with locally excited states or the ground state. For a pure charge transfer state $C(r)$ is approximately 0.09 eV [18]. Substitution of these values into the equation gives an emission maximum at about 490 nm. While the position of the maximum of the weak longer wavelength phosphorescence observed from GpC in EW cannot be determined with precision, the band is in a region where the equation predicts that a guanyl–cytidyl charge transfer state would emit.

References

1 M. Gueron, J. Eisinger and R. G. Shulman, *J. Chem. Phys.*, *47* (1967) 4077.
2 J. Eisinger and R. G. Shulman, *Science*, *161* (1968) 1311.
3 J. Eisinger, M. Gueron, R. G. Shulman and T. Yamane, *Proc. Natl. Acad. Sci. U.S.A.*, *55* (1966) 1015.
4 V. Kleinwachter and J. Koudelka, *Collect. Czech. Chem. Commun.*, *37* (1972) 3433.
5 C. Helene and A. M. Michelson, *Biochim. Biophys. Acta*, *142* (1967) 12.
6 K. Imakubo, *J. Phys. Soc. Jpn.*, *24* (1968) 1124.
7 M. W. Warshaw and I. Tinoco, *J. Mol. Biol.*, *13* (1965) 54.
8 O. Sinanoglu and S. Abdulnur, *Photochem. Photobiol.*, *3* (1964) 333.
9 W. H. Melhuish, *Appl. Opt.*, *14* (1975) 26.
10 W. H. Melhuish, unpublished results, 1980.
11 W. H. Melhuish, *J. Res. Natl. Bur. Stand., Sect. A*, *76* (1975) 547.
12 J. B. Birks, *Photophysics of Aromatic Molecules*, Wiley, New York, 1970.
13 W. Rhodes, *J. Am. Chem. Soc.*, *83* (1961) 3609.
14 W. W. Hauswirth and M. Daniels, in S. Y. Wang (ed.), *Photochemistry and Photobiology of Nucleic Acids*, Vol. 1, Academic Press, New York, 1976, p. 109.
15 J. P. Morgan and P. R. Callis, *Photochem. Photobiol.*, *29* (1979) 1107.
16 M. W. Warshaw and I. Tinoco, Jr., *J. Mol. Biol.*, *20* (1966) 29.
17 M. W. Warshaw and C. R. Cantor, *Biopolymers*, *9* (1970) 1079.
18 H. Beens and A. Weller, in E. Lim (ed.), *Molecular Luminescence*, Benjamin, New York, 1969, p. 203.
19 M. Varadi, Zs. Feher and E. Pungor, *J. Chromatogr.*, *90* (1974) 259.
20 B. Janik and P. J. Elving, *Chem. Rev.*, *68* (1968) 285.