ON THE PHOSPHORESCENCE OF DNA[1]

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This note will present data on the phosphorescence properties of purines, pyrimidines and DNA. The data suggests a delocalized exciton picture of the triplet state.

The measurements reported here were made with an Aminco-Keirs Spectrophosphorimeter. The substances used were all A grade chemicals of the California Biochemical Corporation, except for potassium polyadenylate (poly A) which was grade B and polyuridylic acid (poly U) which was grade C. The solutions used were made in water and then diluted with glycerol to make a final concentration of 3×10^{-4} M in nucleotides in a solvent that was 5% water and 95% glycerol. Spectra were taken at 77°K.

No emission was observed from the following: deoxycytidylic acid, uracil, cytosine, thymine and poly U. Since the instrument used could not detect emission with a half life of less than 10^{-2} secs we may say that either pyrimidines do not emit or that they have half lives less than 10^{-2} secs.

Guanine derivatives phosphoresce and all show about the same spectral features with a characteristic decay time of 1.2 secs (Fig.1). Adenine derivatives also phosphoresce and all show about the same spectral features with a characteristic decay time of about 2.3 secs (Fig.2).

DNA shows an emission which appears to be a simple sum of a guanine and adenine type (Fig.3). The DNA emission can be shown to be composite by

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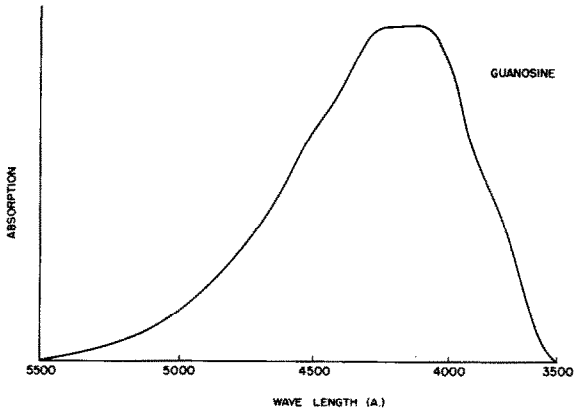

Fig. 1. Phosphorescence of guanosine.

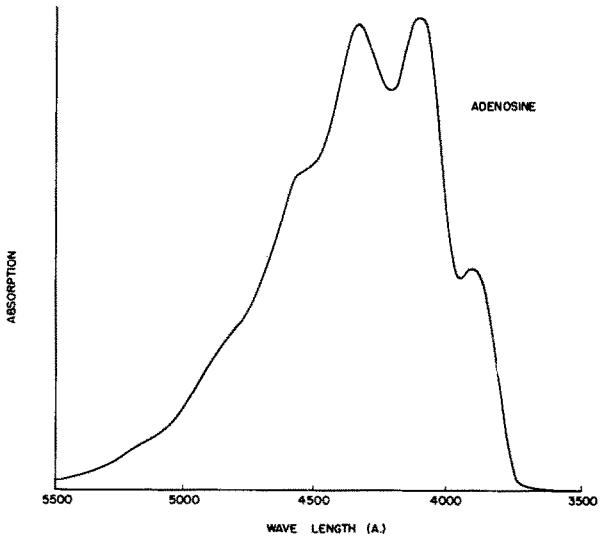

Fig. 2. Phosphorescence of adenosine.

Measuring the decay time at different wavelengths. At 4000 Å and 5000 Å the lifetimes are 1.9 secs and 0.7 secs. This suggests that the guanine and adenine bases in DNA emit independently.

The long-lived emission of DNA is quenched by Mn^{++} and Fe^{+++}. The quenching is non-stoichiometric. Mn^{++} or Fe^{+++} added to DNA, so that the purine-cation ratio is ten, will reduce the emission to below the noise level of the instrument.

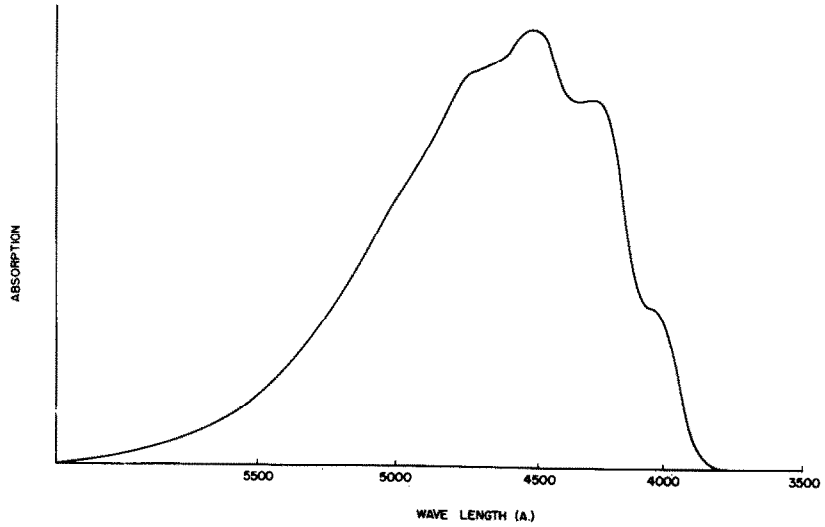

Fig. 3. Phosphorescence of DNA.

Nuclear magnetic resonance studies (Eisinger et al, 1962) have indicated that the bulk of the Mn^{++} or Fe^{+++} bound to DNA is linked to phosphate. In this position the ions cannot quench the triplet state. Only ions that have entered the helix can be expected to quench. The ratio of purines quenched to quenching ions is therefore greater than ten and may actually exceed ten by several orders of magnitude.

It should be noted that a glass of DNA and Mn^{++} with a base pair - Mn^{++} ratio of 10 showed undiminished fluorescence but no phosphorescence. It should also be noted that at an adenine - Mn^{++} ratio of 10 the Poly A phosphorescence is severely quenched but the deoxyadenosine monophosphate phosphorescence is very little reduced.

We conclude that the evidence favors the idea of a delocalized triplet state in DNA.

The above results bring to mind the observation of Steele and Szent-Györgyi (1957) that indicated that benzpyrene might quench the emission of DNA non-stoichiometrically.

It should be pointed out that all of the observations reported here were made on glasses that are cracked at 77°K. Furthermore, at times, impuri-

ties in the liquid nitrogen bath surrounding the sample could appreciably scatter light from the optical paths of the phosphorimeter. The quenching ratios reported must therefore be considered as semi-quantitative only. Work aimed at improving the quantitative aspects of these studies is now in progress. A more complete account and analysis of the results to date will be published elsewhere.

References

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