FLUORESCENCE OF SYNTHETIC DNA's AT ROOM TEMPERATURE AND NEUTRAL pH

James E. Gill

Bio-Medical Division, Lawrence Radiation Laboratory,

University of California, Livermore, California 94550

Received June 14, 1971

Summary: The fluorescence of two synthetic DNA's, polyd(m^5C) and poly[d(I-m^5C)] is demonstrated at room temperature and neutral pH. Polyd(m^5C) at pH 8.0 exhibits fluorescence qualitatively the same as the mononucleotide: the quantum yield is independent of excitation energy; the emission maximum is at 2.92 μ^{-1} (355 nm). Poly[d(I-m^5C)] exhibits fluorescence resembling that of the 5-methyldeoxycytidine component with an additional feature that is probably due to weak deoxyinosine fluorescence. Neither of these synthetic DNA's exhibits spectra suggestive of exciplex formation.

INTRODUCTION

Encouraged by the observations of fluorescence of DNA at low temperature (1, 2) and desirous of obtaining room-temperature fluorescence to serve as a probe for radiation damage and to test the applicability of low-temperature data to room-temperature conditions, I have attempted to identify fluorescent analogs of nucleic-acid derivatives which could be enzymatically incorporated into synthetic DNA's and could serve as fluorescent probes of DNA excited states. It has now become possible to incorporate the weakly fluorescent minor nucleoside, 5-methyldeoxycytidine (3) into alternating copolymers and homopolymer pairs. This communication contains the first report of luminescence from such synthetic DNA molecules at room temperature and neutral pH.

MATERIALS AND METHODS

5-Methyldeoxycytidylic acid purchased from P-L Biochemical Co. was converted to the triphosphate by International Chemical and Nuclear Corporation. Deoxyinosine trisphosphate was purchased from Sigma Chemical Co. Thin-layer chromatography revealed both compounds to be free of gross contaminants. Polyd(m^5C)* was obtained by separation of strands of polyd(G) : polyd(m^5C) which had been synthesized with partially purified E. coli DNA polymerase according to Burd and Wells (4). The copolymer of deoxyinosine and 5-methyldeoxycytidine, poly[d(I-m^5C)], was synthesized according to Burd and Wells. This product is tentatively identified as the copolymer because (a) it formed a single band in alkaline CsCl; (b) it melted at 44°C in .01 M KCl, pH 6.9; (c) the absorption spectrum has features due to inosine and 5-methylcytosine; (d) repeated attempts to synthesize polyd(I) · polyd(m^5C), the most likely contaminant, have been unsuccessful. Burd and Wells report no evidence of enzymatic reaction products other than the copolymer or the homopolymer pair.

Following ethanol precipitation and redissolution, the polymers were purified by banding on a neutral CsCl gradient and by dialysis. The copolymer, poly[d(I-m^5C)], was further purified by repeated membrane dialysis over an Amicon PM-10 Diaflow membrane.

Fluorescence emission spectra were obtained with an Aminco-Bowman spectrophotofluorometer. Fluorescence excitation spectra were obtained and corrected using an instrument and techniques already described (3, 5).

All pH measurements were made at room temperature with a Corning Model 10 pH meter.

*Abbreviations are those of the IUPAC-IUB Commission on Biochemical Nomenclature.

RESULTS

Polyd(m^5C) at pH 8.0 and 20 °C exhibits fluorescence spectra resembling those of the mononucleotide: the emission maximum is at 2.92 ± .04 μ^{-1} (355 nm); the emission spectrum of polyd(m^5C) is independent of excitation energy. Corrected excitation and absorption spectra match closely throughout the range from 3.10 to 4.20 μ^{-1} (240 to 310 nm) (Fig. 1).

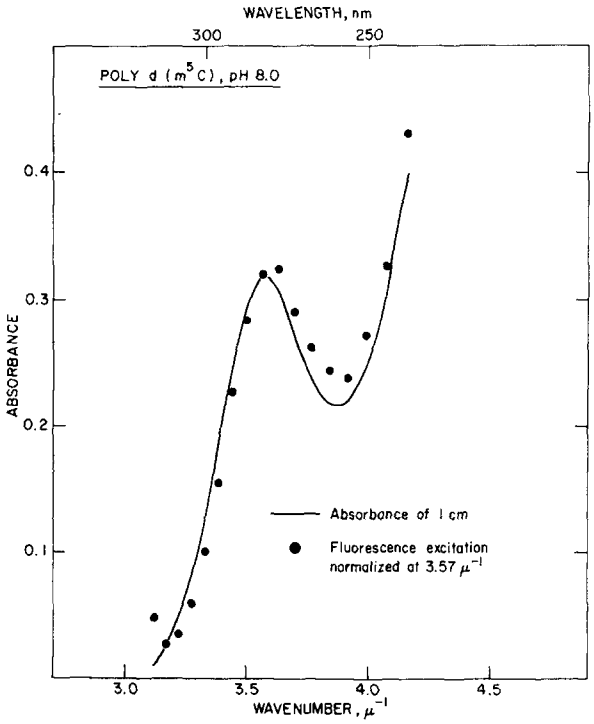

Fig. 1. Absorption spectrum and corrected fluorescence excitation spectrum of polyd(m^5C) in 0.005 M Tris-HCl, pH 8.0, 20 °C. The instrumental bandwidth for the excitation spectrum was 0.033 μ^{-1} (2.1 nm) at 3.93 μ^{-1}.

Poly[d(I-m^5C)] also exhibits luminescence which emanates predominately from the 5-methylcytosine bases. The emission maximum is at 2.93 ± .04 μ^{-1} and the corrected excitation spectrum has a maximum at 3.55 μ^{-1}, close to the absorption maximum for polyd(m^5C). In contrast to the polyd(m^5C) excitation and absorption spectra, which have a minimum

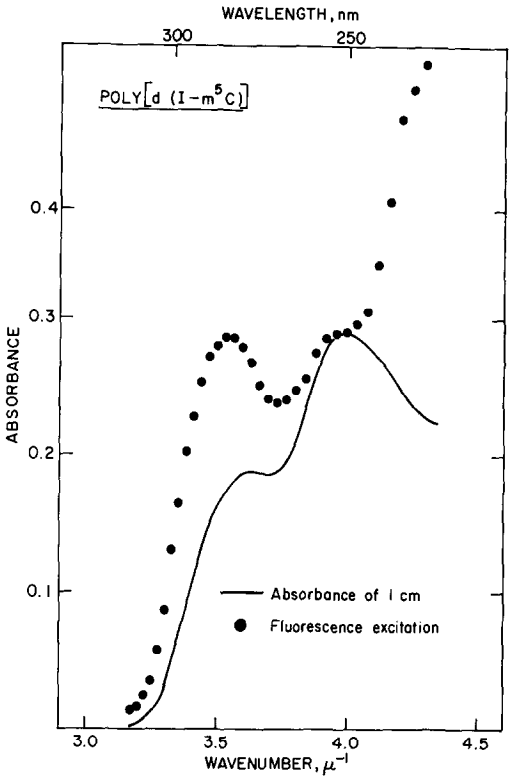

Fig. 2. Absorption spectrum and corrected fluorescence excitation spectrum of poly[d(I-m^{5}C)] in 0.02 M KCl + 0.005 M PIPES buffer, pH 6.85, 20°C. Instrumental bandwidth for the excitation spectrum was 0.036 μ^{-1} (2.3 nm) at 3.93 μ^{-1}.

---

at 3.93 μ^{-1}, the poly[d(I-m^{5}C)] excitation spectrum has a shoulder at this energy which corresponds to an absorption maximum of deoxyinosine (Fig. 2). The emission spectrum of poly[d(I-m^{5}C)] does depend weakly upon excitation energy: the ratio of emission intensity at 2.60 μ^{-1} to that at 2.93 μ^{-1} is greater when the excitation energy corresponds to the deoxyinosine absorption maximum than it is when the excitation is at the 5-methyldeoxycytidine maximum (Table I).

DISCUSSION

Polyd(m^{5}C), a random coil at pH 8.0 (6), exhibits fluorescence spectra which are similar to those of the monomer. There is little if any (< 0.05 μ^{-1})

Table I

Poly[d(I-m^{5}C)] Fluorescence Emission Spectral Ratios

Excitation Wave Number	F (2.60)/F (2.93)*
3.55 μ^{-1}	0.54
3.93 μ^{-1}	0.80

*These ratios pertain to uncorrected fluorescence emission spectra.

shift of the emission maximum, which would be expected upon excimer formation; there is little if any fluorescence quenching. The base-base interaction, which gives rise to the hypochromism of random-coil polyd(m^{5}C) relative to the monomer (6), does not qualitatively change the monomer fluorescence. The close agreement between corrected excitation and absorption spectra argues against increased intersystem crossing with increasing excitation energy, as has been reported (7, 8, 9, 10) for uracil, orotic acid, and thymine.

The incorporation of 5-methyldeoxycytosine and deoxyinosine into a double helical structure also does not substantially quench or alter the 5-methyldeoxycytosine fluorescence. The emission spectrum of poly[d(I-m^{5}C)] resembles that of poly(dm^{5}C) and 5-methyldeoxycytosine; I could detect no shift that might indicate exciplex formation, and the intensities are qualitatively the same. These results are consistent with those of Pochon et al. (11), who found that the fluorescence of 7-methylinosine persists when the nucleoside is incorporated into poly(I, m^{7}I) · poly(C). In contrast, Ward et al. (12) reported that the incorporation of formycin, 2-aminopurine, or 2, 6-deaminopurine into a double-stranded helical copolymer with a uracil derivative decreased the fluorescence quantum yield to approximately 0.1 to 1.5% of the

corresponding free monomers. Also, formation of hydrogen bonds between guanine and 1-methyl-N_4-acetylcytosine quenches the fluorescence of the latter at 77°K (13). It does not appear possible to predict when hydrogen bonding of nucleotides or their incorporation into a helix will quench their fluorescence.

The excitation spectrum of poly[d(I-m^{5}C)] resembles that of polyd(m^{5}C) except for the shoulder at 3.93 μ^{-1}, the deoxyinosine absorption maximum. The shape of this spectrum implies that most of the fluorescence is caused by direct excitation of 5-methyldeoxycytidine residues. The shoulder at 3.93 μ^{-1} has several possible explanations: (a) Singlet energy transfer from deoxyinosine to 5-methyldeoxycytidine, (b) enhanced 5-methyldeoxycytidine absorbance at this energy, (c) impurity fluorescence, (d) deoxyinosine fluorescence. The persistance of this shoulder after membrane dialysis argues against (c). If (a) or (b) were correct, there would be no change in the emission spectrum as the excitation energy varied. However, if (d) were correct, excitation at 3.93 μ could be expected to shift the emission spectrum, assuming deoxyinosine and 5-methyldeoxycytidine emitted at different energies. The observed shift thus supports (c) as the correct explanation of the excitation shoulder at 3.93 μ^{-1}. Inosine fluorescence has been reported (14).

The results presented here show that 5-methylcytosine fluorescence will be a useful probe of DNA excited states for such phenomena as energy transfer, exciplex formation, fluorescence quenching, and perhaps biprotonic phototautomerism (15). None of these have been observed in random polyd(m^{5}C) or in poly[d(I-m^{5}C)], but these results may very well not hold for other 5-methylcytosine-containing DNA. Spectroscopic properties of "acid" polyd(m^{5}C) and of poly[d(G-m^{5}C)] are currently under study.

ACKNOWLEDGMENTS

I thank C. Bishop for synthesis of the polynucleotides, J. Mazrimas

for measurement of melting and buoyant density, and R. L. Baldwin for helpful discussions. This work was performed under the auspices of the U.S. Atomic Energy Commission. Reference to a company or product name does not imply approval or recommendation of the product by the University of California or the U.S. Atomic Energy Commission to the exclusion of others that may be suitable.

REFERENCES

1. Eisinger, J., and Shulman, R.G., Science 161, 1311 (1968).
2. Eisinger, J., Lamola, A.A., Longworth, J.W., and Gratzer, W.B., Nature 226, 113 (1970).
3. Gill, J.E., Photochem. Photobiol. 11, 259 (1970).
4. Burd, J.F., and Wells, R.D., J. Mol. Biol. 53, 435 (1970).
5. Gill, J.E., Photochem. Photobiol. 9, 313 (1969).
6. Zmudzka, B., Bollum, F.S., and Shugar, D., Biochemistry 8, 3049 (1969).
7. Brown, I., and Johns, H.E., Photochem. Photobiol. 8, 273 (1968).
8. Whillans, D.W., and Johns, H.E., Photochem. Photobiol. 9, 323 (1969).
9. Fisher, G.J., and Johns, H.E., Photochem. Photobiol. 11, 429 (1970).
10. Hauswirth, W., and Daniels, M., Photochem. Photobiol. 13, 157 (1971).
11. Pochon, F., Leng, M., and Michelson, A.M., Biochim. Biophys. Acta 169, 350 (1968).
12. Ward, D.C., Reich, E., and Stryer, L., J. Biol. Chem. 244, 1228 (1969).
13. Hélène, C., Biochem. Biophys. Res. Commun. 22, 237 (1966).
14. Walaas, E., Acta Chem. Scand. 17, 461 (1963).
15. Taylor, C.A., El-Bayoumi, M.A., and Kasha, M., Proc. Natl. Acad. Sci. (U.S.) 63, 253 (1969).