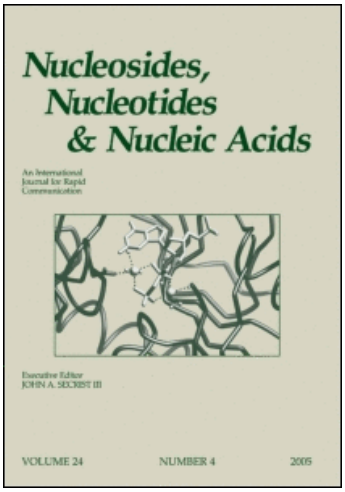

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The Fluorescence Property of Dinucleoside Monophosphates Containing Ethenoadenosine and 5, 6-Dihydrouridine Derivatives

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THE FLUORESCENCE PROPERTY OF DINUCLEOSIDE MONOPHOSPHATES CONTAINING ETHENOADENOSINE AND 5,6-DIHYDROURIDINE DERIVATIVES

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ABSTRACT: The role of neighboring nucleotides in the quenching of etheno-adenosine fluorescence as a constituent of dinucleotides is studied. Bisulfite reacts with the uracil moiety of dinucleoside monophosphate, UpεA to yield an addition product, 5,6-dihydro-6-sulfouridylyl-ethenoadenosine. During the course of the reaction, the fluorescence of ethenoadenosine increases up to 8-fold, to the same intensity as that of monomeric ethenoadenosine. 5,6-Dihydrouridylyl-ethenoadenosine has a similar fluorescence intensity. These results form evidence that the role played by a neighboring nucleotide in the quenching of ethenoadenosine fluorescence in oligo- or polynucleotides is to generate an aromatic-aromatic interaction between these bases.

The fluorescence intensity of etheno-(deoxy)adenosine in dinucleotides and longer oligo-(poly)nucleotides is about one tenth of that in the monomeric form. This quenching of fluorescence has been believed to be caused by the base-base stacking interactions. Therefore, etheno-(deoxy)adenosine in oligonucleotides and polynucleotides has been used as a sensitive probe for base-base stacking interactions.[1,2] The binding of recA protein to polynucleotides containing ethenodeoxyadenosine enhances their fluorescence greatly and therefore this effect is used for measurement of the binding of recA protein to nucleic acids.[3] The mechanism with which recA protein abolishes the quenching of the fluorescence would be important, because this effect may be

§ This paper is dedicated to Professor Morio Ikehara on the occasion of his 70th birthday.

closely related to activities of recA protein to induce recombination. A knowledge of the nature of the fluorescence quenching by a neighboring nucleoside is essential for the elucidation of the mechanism of this effect. However, it is not known whether the fluorescence is quenched only by the presence of a neighboring base having a planar aromatic ring. Here we have compared the fluorescence property of dinucleoside monophosphates containing ethenoadenosine (εA) and dihydrouridine (D) to that of UpεA.

MATERIALS AND METHODS

UpεA was prepared from UpA with bromoacetaldehyde treatment. Bromoacetaldehyde reacts with adenosine to yield ethenoadenosine.[4] DpεA was prepared from DpA similarly with bromoacetaldehyde treatment. DpA was obtained as follows. UpA was hydrogenated with H_2 gas in the presence of Rh on alumina.[5] The resulting mixture of UpA and DpA was treated with bisulfite. Uridine in UpA was converted to 5,6-dihydrouridine 6-sulfonate (U*) while DpA was not affected.[6] DpA was separated from U*pA with paper chromatography (solvent I, 1-butanol-acetic acid-water, 2:1:1 by vol.) and rechromatography (solvent II, isobutyric acid: 0.5 N NH_4 OH, 5:3 v/v). DpA thus obtained was about 90 % pure. Absorption spectra of UpεA and DpεA are shown in FIG. 1.

The concentrations of dinucleotides and εAp were estimated from the absorbance at 310 nm ($\varepsilon = 2.0 \times 10^3$) on the basis of the fact that Up, U*p and Dp show no absorption at this wavelength. Fluorescence emission spectra were recorded at pH 6.7-6.9 with a Hitachi MPF-4 fluorescence spectrophotometer. The excitation was made at 310 nm. Fluorescence intensity was measured at its emission maximum (407 nm) unless otherwise stated.

RESULTS AND DISCUSSION

UpεA was treated with 0.5 M sodium bisulfite at pH 6.9 and 37°. Aliquots were diluted with 0.05 M Na-phosphate, pH 6.7. The absorption spectra and fluorescence emission spectra were measured. Results are shown in FIG. 2. Bisulfite treatment converts UpεA to U*pεA. In accordance with the decrease of ultraviolet absorption, fluorescence intensity increased. After 1.5-2 hr, the fluorescence intensity reached a value 7.3 times that of the starting UpεA. U* is known to revert to uridine in alkaline solutions. If the increase in fluorescence intensity was due to the conversion to U*pεA, the quenching of the fluorescence should be restored by subsequent treatment

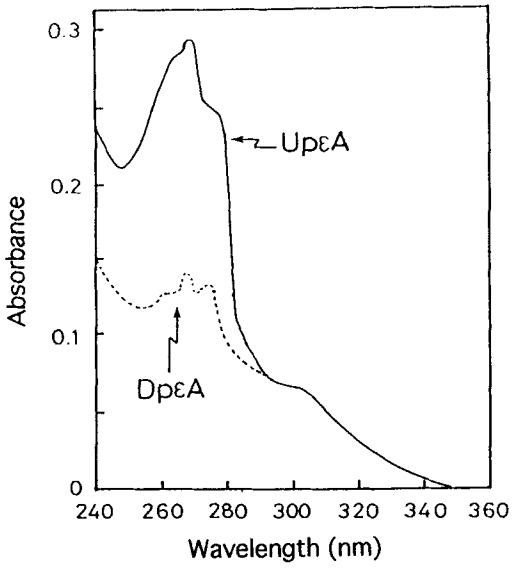

FIG. 1. Absorption spectra of UpεA and DpεA

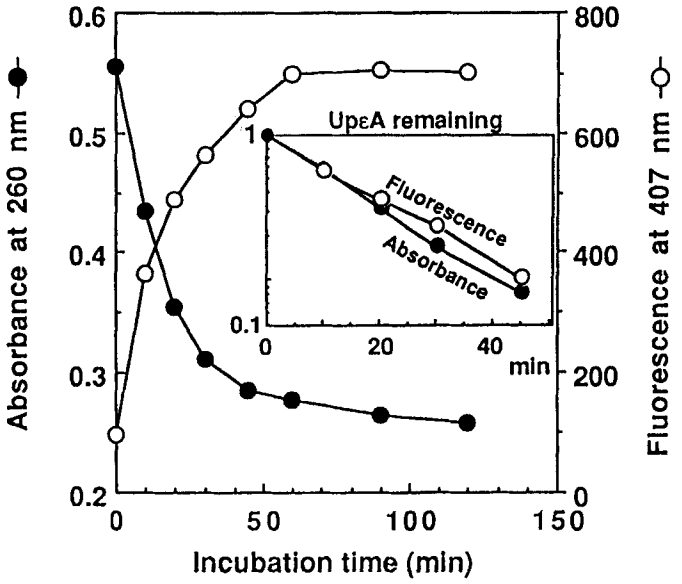

FIG. 2. Time course of conversion of UpεA to U*pεA with bisulfite. The inset shows the amounts of UpεA remaining in the reaction mixtures as calculated from the increases in fluorescence, or decreases in the absorbance.

TABLE 1. Relative fluorescence intensity of εA-containing nucleotides

	εAp	UpεA	U*pεA	DpεA
	1	0.12	1.04	1.05
After RNase T_2 digestion[a)]	-	0.93	1.02	1.13

a) With this RNase T_2 treatment, 96 % of UpεA, 70 % of U*pεA and 80 % of DpεA were digested into monomeric components.

with mild alkali. The reaction mixture containing U*pεA was diluted 100-fold with 0.02 M Tris-HCl, pH 8.9, and allowed to stand at room temperature (25°). After 1.06, 3.8 and 5.7 hr, the fluorescence intensity decreased to 61, 28 and 18.6 % of the original value respectively. In TABLE 1, the fluorescence intensities of purified U*pεA and DpεA are shown as compared to those of UpεA and εAp. The intensities for DpεA and U*pεA were the same as that of εAp. They were 8.7-fold greater than that of UpεA. On digestion of UpεA with RNase T_2, the fluorescence intensity increased markedly, whereas the intensity of DpεA and U*pεA was not affected by the digestion. These results showed the absence of fluorescence quenching in U*pεA and DpεA. Thus, we gave evidence experimentally that the quenching of εA-fluorescence in dinucleotides occurs by interactions of the εA ring with the heteroaromatic ring in the adjacent nucleotide.

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