Biophysical Chemistry 9 (1979) 105–109

FLUORESCENCE STUDIES OF THE INTERACTION BETWEEN 1,N^6-ETHENOADENOSINE MONOPHOSPHATE AND NUCLEOTIDES

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Received 10 April 1978

AMP, GMP, TMP and CMP quench the fluorescence of 1,N^6-ethenoadenosine monophosphate (ϵ-AMP). The fluorescence spectrum of ϵ-AMP-nucleotide system is identical with that of ϵ-AMP itself, and the fluorescence decay kinetics follow a single-exponential decay law. The dependence of fluorescence yields and lifetimes upon the concentration of nucleotides shows that the fluorescence of ϵ-AMP is principally quenched in a dynamic process by AMP, TMP and CMP, while it is quenched in both dynamic and static processes by GMP. The quenching constants increase in the following order: GMP > AMP > TMP > CMP.

1. Introduction

Fluorescent modification of the base residues in nucleic acids is of importance for structural and functional investigation of RNA and DNA [1]. Barrio et al. [2] found that chloroacetaldehyde reacts with adenosine and cytidine derivatives to form highly fluorescent products. Because of stability, high fluorescence yields and long fluorescence lifetimes of ϵ-adenosine and its nucleotides [1–3], they may prove useful in biochemical and biophysical studies of nucleic acids. Steiner and co-workers [4] have shown the possibility of studying polynucleotides containing chloroacetaldehyde-modified ϵ-adenosine groups by using the polarization of fluorescence. It has recently been reported by Wetmur et al. [5,6] that denatured DNA can react with chloroacetaldehyde to produce fluorescence properties similar to ϵ-AMP. The fluorescence yield of ϵ-DNA has been found to be very small compared to that of free ϵ-AMP [5,6]. The result implies that DNA bases may quench the fluorescence of ϵ-AMP. In the present work we have studied the effect of nucleotides on the fluorescence of ϵ-AMP, aiming to understand the interactions of ground and excited states of ϵ-AMP with nucleotides.

2. Materials and methods

ϵ-AMP was synthesized and purified according to the method of Secrist et al. [3]. Nucleotides, chromatographically pure, were obtained from Sigma Chemical Co. or Seikagaku Kogyo.

Absorption spectra were measured with a Shimadzu UV-200S spectrophotometer.

Fluorescence quantum spectra were measured with a Hitachi MPF-2A spectrophotofluormeter calibrated by using a standard tungsten lamp. Fluorescence quantum yields were determined according to the method of Parker and Rees [7]; quinine sulfate in 1 N H_2SO_4 was used as the standard reference [8].

Fluorescence decay curves were measured with an ORTEC Model 9200 nanosecond fluorescence spectrophotometer interfaced with a PDP 11/04 computer (Digital Equipment Corp.). Excitation light was passed through a grating monochromator (Applied Photophysics Ltd.) and focussed on the sample with a lens. Light emitted from the sample was passed through appropriate interference (Japan Vacuum Optics) and cut-off filters (Toshiba). In the present study, the excitation and emission wavelengths were, respectively, 337 and 410 nm. A dilute solution of colloidal silica (Ludox, Dupont) was used to determine the exciting lamp flash profile. The flash lamp (Applied Photophysics Ltd.) was thyratron-triggered, air-filled (0.5

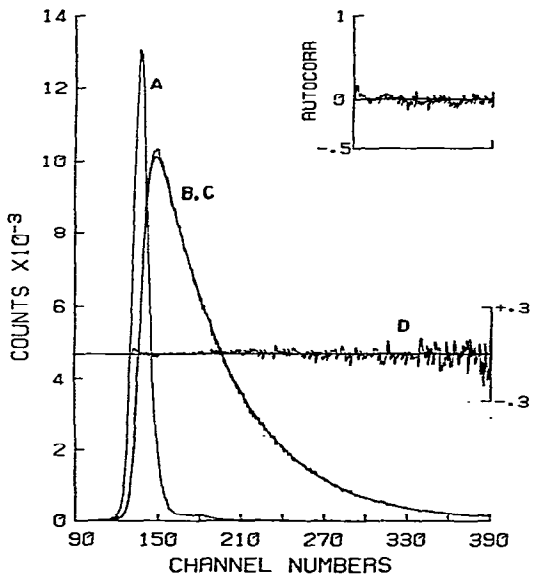

Fig. 1. Experimental and computed fluorescence decay curves of ε-AMP (1.0×10^{-4} M) in 0.1 M phosphate buffer (pH 6.8) at 25°C. Curve A is the lamp flash profile. Curve B is the experimental decay curve. Curve C, superimposed on Curve B, shows the single component analysis. Curve D is the percent residuals. The inset is the autocorrelation function of the residuals. Timing calibration was 0.453 ns/channel. The excitation and emission wavelengths were, respectively, 337 and 410 nm. Parameters obtained: τ = 23.6 ns, χ^2 = 1.22.

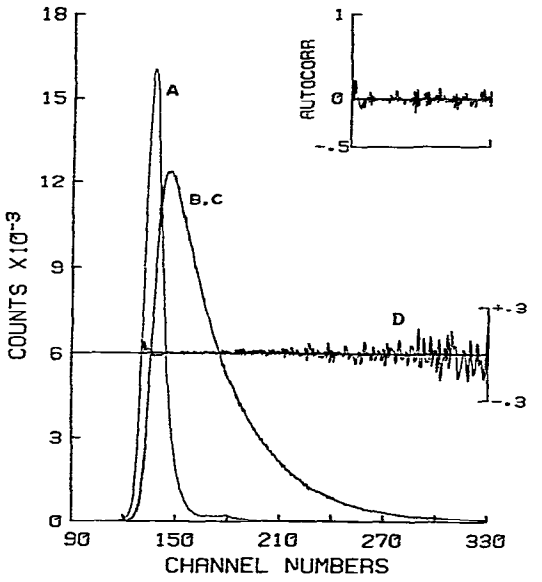

Fig. 2. Experimental and computed fluorescence decay curves of ε-AMP (1.0×10^{-4} M) in the presence of GMP (0.02 M) in 0.1 M phosphate buffer (pH 6.8) at 25°C. The experimental conditions are the same in fig. 1. Parameters obtained: τ = 14.3 ns, χ^2 = 1.33.

atm), and run at about 30 kHz. The decay data were analyzed with the aid of the methods of non-linear least-squares [9] and Laplace transforms [10]. Both methods yielded very similar results. The parameters obtained by analysis were convolved with the experimental lamp flash and the fit between the experimental and computed decay curves was evaluated by the inspection of the weighed residuals, the autocorrelation function of the residuals and the reduced χ^2 [9–11].

All the measurements were carried out in 0.1 M phosphate buffer (pH 6.8) at room temperature (25 ± 1°C).

3. Results and discussion

It was found in the present study that the four nucleotides, AMP, GMP, TMP and CMP, quench the fluorescence of ε-AMP. While the fluorescence intensity of ε-AMP was decreased in the presence of nucleotides, its fluorescence spectrum was identical with that of ε-AMP alone.

Next, the fluorescence decay curves were obtained by the single photon counting technique. Typical fluorescence decay curves are shown in figs. 1 and 2. Over a wide concentration range of nucleotides, the decay curves were purely single exponential and provided no evidence of a $t^{-1/2}$ dependence which would be introduced by the transient term in the fluorescence quenching rate constant [12]. The fluorescence lifetimes (τ) and χ^2 values obtained for a single-exponential decay law are shown in table 1. The fluorescence lifetime of ε-AMP (23.6 ns) is in good agreement with the value (23.8 ns) measured in 0.1 M KH_2PO_4 (pH 6.8) at 23°C by the phase and modulation method [13].

Table 1
Fluorescence lifetimes of ε-AMP * in the presence of nucleotides

Conc (M)	GMP		AMP		TMP		CMP	
	τ (ns)	χ^2	τ (ns)	χ^2	τ (ns)	χ^2	τ (ns)	χ^2
0.005	19.5	1.52						
0.0075			19.2	1.25				
0.010	17.5	1.20			18.2	1.71	19.7	1.39
0.015	15.6	1.49	16.5	1.45				
0.020	14.3	1.33			15.4	1.61	17.5	1.18
0.0225			14.9	1.45				
0.030			13.1	1.72	13.1	1.66	15.7	1.57
0.040					11.4	1.49	14.4	1.44

* The values of τ and χ^2 for ε-AMP were, respectively, 23.6 ns and 1.22.

Stern-Volmer plots are shown in fig. 2. Here, ϕ_0 and ϕ are fluorescence quantum yields in the absence and presence of quencher, and τ_0 (23.6 ns) and τ are fluorescence lifetimes in the absence and presence of quencher. The value of ϕ_0, measured relative to a quinine sulfate standard taken as 0.55 [8], is 0.52. Both plots of ϕ_0/ϕ and τ_0/τ against the concentration of quencher are linear and coincide well with each other over a wide concentration range except for GMP. The plots of ϕ_0/ϕ at a high concentration range except for GMP deviate from linearity. The deviation however is not caused by trivial optical effects because absorption of GMP at the excitation wavelength (335 nm) and its fluorescence were negligible.

In general, the fluorescence quenching data can be analyzed according to the following kinetic scheme [14,15]:

$$\begin{array}{ccc} A^* + Q & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & (AQ)^* \rightarrow \text{quenching} \\ \Big\uparrow\Big\downarrow & & \Big\uparrow\Big\downarrow \\ A + Q & \underset{}{\overset{K_a}{\rightleftharpoons}} & AQ \end{array}$$

In view of findings mentioned above, it seems likely that (AQ)* is non-fluorescent in the present case. By assuming strong quenching ($k_{-1}\tau_0^c \ll 1$) where k_{-1} is the dissociation rate constant of (AQ)* and τ_0^c is the fluorescence lifetime of the complex AQ, we can obtain the following equations [15]

$$\phi_0/\phi = (1 + K_a[Q])(1 + k_1\tau_0[Q]), \quad (1)$$

$$\tau_0/\tau = 1 + k_1\tau_0[Q], \quad (2)$$

where $K_a = [AQ]/[A][Q]$ is the molar association constant for the ground-state complex formation and k_1 is the rate constant of fluorescence quenching of A* by encounters with quencher.

Positive deviations from the Stern-Volmer law in the case of GMP can be attributed to the existence of two dinstict quenching mechanisms, dynamic and static quenching ones [14,15]. The curve in fig. 2b was analyzed according to eq. (1); the results gave association constant K_a of 4.0 M^{-1} for formation of a non-fluorescent ground-state complex between ε-AMP and GMP. This value is comparable with the association constants for the formation of ground-state complexes between DNA bases [16]. Penzer and Robertson [17] found that the plots of ϕ_0/ϕ for AMP display downward curvature at a high concentration range (above 0.05 M). They attributed this phenomenon to self-association of AMP and the subsequent decrease of its activity coefficient as its concentration is raised [17]. In this study, this phenomenon was not observed presumably due to a lower concentration range of AMP (below 0.04 M).

It is seen from fig. 2 that

$$\phi_0/\phi = \tau_0/\tau \quad (3)$$

for AMP, TMP and CMP over a wide concentration range. This result implies that these nucleotides principally operate by the dynamic quenching mechanism [15]. By using the value of τ_0 (23.6 ns), the rate constants of fluorescence quenching (k_1) listed in table 2 have been calculated. These values show that the quenching process is a diffusion-controlled one [18].

Table 2
Quenching constants at pH 6.8 and 25°C

Quencher	$k_1\tau_0$ (M^{-1})	k_1 (M^{-1} ns^{-1})	Ionization potential (eV) *
GMP	33.5	1.42	–
AMP	27.5	1.17	8.91
TMP	26.0	1.10	9.43
CMP	16.5	0.70	8.90

* The values of the corresponding bases, quanine, adenine, thymine and cytosine (ref. [22]).

Tolman et al. [19] found that the fluorescence of 1,N^6-ethenoadenosine in the dinucleotide phosphate is greatly quenched in both dynamic and static processes by the neighboring nucleosides. In the present work, static quenching is observed only with GMP which has a great tendency to form aggregates [16]. Further, it is of interest to notice that the association constant between ε-AMP and GMP is almost the same as the intramolecular association constant obtained with dinucleotides, 1,N^6-ethenoadenyl-(3′→5′)-guanosine and guanylyl-(3′→5′)-ethenoadenosine [19].

Since Weller [20] found that the quenching constants, on the quenching of the acridine fluolrescence by amines, increase with increasing ionization potential of amines, a charge-transfer mechanism has been successful in explaining the quenching of fluorescence [21]. Table 2 lists the values of ionization potential obtained with the corresponding bases, guanine, adenine, thymine and cytosine [22]. The data for guanine are, unfortunately, missing. However, a strong electron-donor ability of guanine is expected by its easy charge-transfer complexation with chloranil [23]. The molecular orbital calculations also show that guanine acts as the best π-donor among the four bases [24,25]. As is seen in table 2, the quenching constants increase in the following order: GMP > AMP > TMP > CMP. This order seems to be in line with the order of electron-donor ability of the base except for CMP. The reason why CMP behaves in ways different from other nucleotides is not clear from this work.

In conclusion, steady-state fluorescence intensity and transient decay measurements clearly indicate that the fluorescence of ε-AMP is principally quenched in a dynamic process by AMP, TMP and CMP, while it is quenched in both dynamic and static processes by GMP.

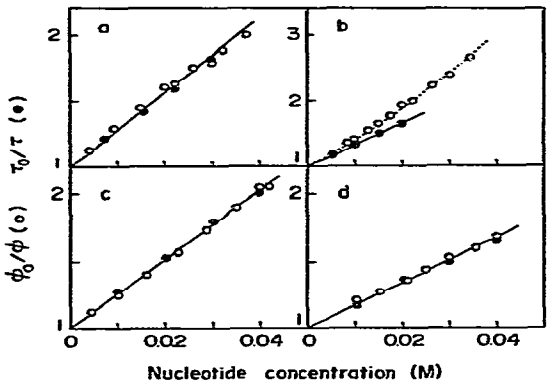

Fig. 3. Stern-Volmer plots for (a) ε-AMP-AMP, (b) ε-AMP-GMP, (c) ε-AMP-TMP and (d) ε-AMP-CMP systems. ϕ, ϕ_0, τ and τ_0 are defined in the text. The solvent was 0.1 M phosphate buffer (pH 6.8) at 25°C. The concentration of ε-AMP was 1.0×10^{-4} M. For measurements of fluorescence spectra, the excitation wavelength was 335 nm except for ε-AMP-AMP system (330 nm).

Acknowledgement

We wish to thank Drs. Ludwig Brand and Amiram Grinvald for sending us copies of computer programs on the method of non-linear least-squares.

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