Spectrometric and Biological Data of 1,N^6-Ethenoadenosine 3′,5′-Cyclic Monophosphate

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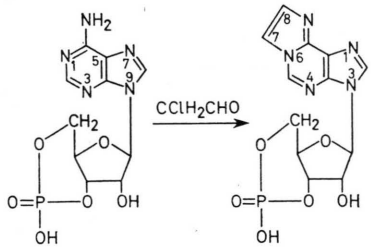
1,N^6-Ethenoadenosine 3′,5′-Cyclic Monophosphate, Fluorescence, Spectra, Protein Kinase

Spectroscopic properties of 1,N^6-ethenoadenosine cyclic 3′,5′-monophosphate were studied and compared with cyclic adenosine 3′,5′-monophosphate. Data of nuclear magnetic resonance-, mass-, ultraviolet-, and fluorescence spectroscopy were given. Additionally biochemical activity was tested in protein kinase system of beef adrenals. No significant biological difference between cyclic adenosine 3′,5′-monophosphate and its fluorescent analog has been found.

Recently it has been shown that chloroacetaldehyde reacts with derivatives of adenine and cytosine to form fluorescent products [1]. For our study 1,N^6-ethenoadenosine 3′,5′-monophosphate (3′,5′-cyclic εAMP) was prepared under conditions earlier described [2]. Homogeneity of product was verified by chromatography on thin layer of silicagel (Merck, Germany) with *n*-butanol, CH_3COOH and H_2O (5 : 2 : 3, v/v/v).



1 2

The numeration of **2** follows according to the nomenclature based on the ring system, 3-β-D-ribofuranosyl-imidazo-(2,1-i) purine 3′,5′-monophosphate.

3′,5′-cyclic AMP was obtained from Boehringer (Mannheim, Germany), (^{3}H)-3′,5′-cyclic AMP from NEN-Chemicals (USA), monochloroacetaldehyde from Fluka (Switzerland). Ultraviolet spectra were obtained on Acta V (Beckman) spectrometer, fluorescence spectra on Zeiss fluorescence spectrometer PM QII with ZFM 4. The NMR-spectra were obtained on Bruker 90 MHz spectrometer with spectra accumulation (Fourier-transformation-impulsspectra) in D_2O, pD adjusted to 6.0 with NaOD, and sodium 3-trimethyl-silylpropionate as internal standard. Mass spectra were obtained by CH 5 (Varian) at 70 eV.

The NMR-spectrum of 3′,5′-cyclic εAMP exhibits a characteristic pair of doublets at δ 7.9 ppm (H-7) and δ 7.5 ppm (H-8) with a spin-coupling constant $J_{7,8} = 2$ Hz. In contrast to the NMR-spectrum of 3′,5′-cyclic AMP, where the protons H-8 and H-2 of purine ring give peaks at δ 8.16 ppm and δ 8.13 ppm in form of two adjacent singlets, in the spectrum of 3′,5′-cyclic εAMP a specific deshielding effect of the

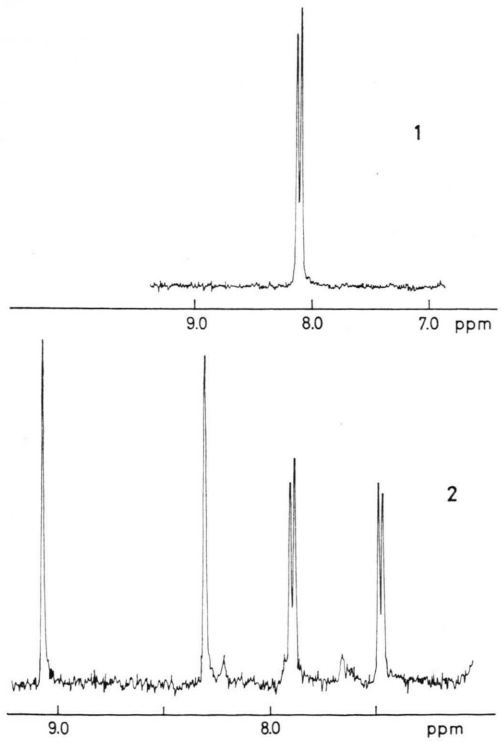


Fig. 1. Characteristic section of 90 MHz Fourier-transform NMR spectrum of 3′,5′-cyclic AMP (**1**) and 3′,5′-cyclic εAMP (**2**).

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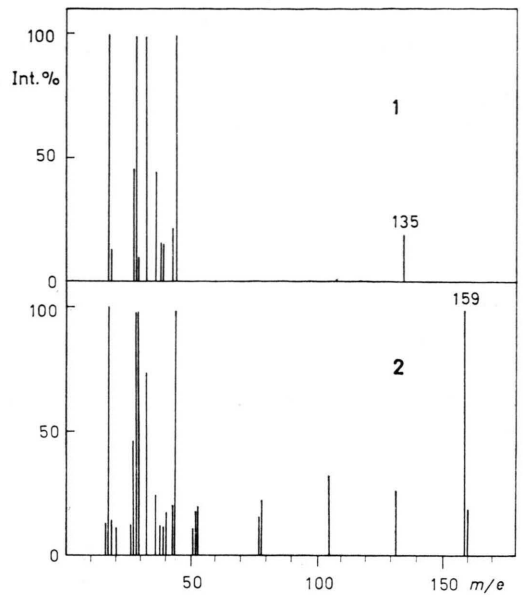
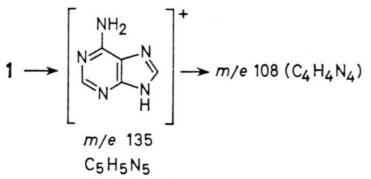


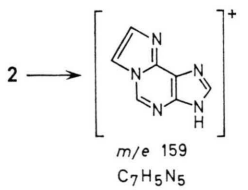
Fig. 2. Mass spectra. 3′,5′-cyclic AMP (**1**) and 3′,5′-cyclic εAMP (**2**).

H-5 (numeration of **2**) was found. H-5 δ 9.016 ppm (s) and H-2 δ 8.28 ppm (s).

In the mass-spectrum of 3′,5′-cyclic AMP (**1**) a characteristic ion was observed at *m/e* 135 ($C_5H_5N_5$ 135.05) corresponding to the adenine fragment ion:



This peak (*m/e* 135) is absent from the mass-spectrum of 3′,5′-cyclic εAMP (**2**). In the mass-spectrum of **2** a new peak (*m/e* 159) of very high intensity can be seen, corresponding to the following fragmentation:



In both cases the formation of these fragments may well occur by a "one-event" fragmentation of 3′,5′-cyclic AMP and 3′,5′-cyclic εAMP.

In the ultraviolet spectrum (Fig. 3) a characteristic red shift of the spectrum of 3′,5′-cyclic εAMP can be seen in contrast to 3′,5′-cyclic AMP and in addition it displays great differences in acidic, neutral and basic solution.

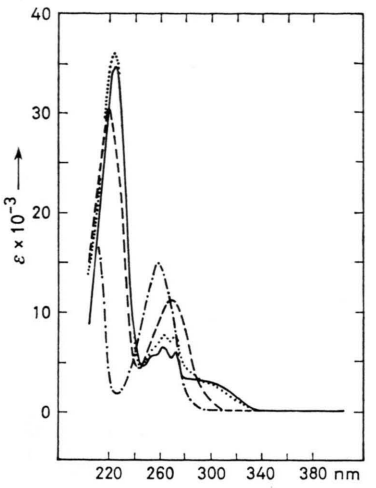


Fig. 3. Ultraviolet spectra. 3′,5′-cyclic AMP (— · — · —) and 3′,5′-cyclic εAMP in neutral aqueous solution (· · · · · ·), in 0.01 N HCl (— — —) and in 0.01 N NaOH (———).

Table I. Ultraviolet absorption data.

3′,5′-cyclic εAMP	λ_{max} [nm]	$\varepsilon \times 10^{-3}$ [M^{-1} cm^{-1}]
in neutral aqueous solution	225	35
	264	7.6
	274	7.6
in 0.01 N HCl	220	30
	273	11
in 0.01 N NaOH	226	34
	264	6.3
	274	5.9

Isosbestic points: 221 nm, 242 nm, 292 nm.

In comparison with other reported spectra of ε analogs[3] our UV-Spectra exhibit additionally two isosbestic points at 242 nm and 221 nm and a strong maximum at 225 nm. The fluorescence emission spectrum of 3′,5′-cyclic εAMP shows the same characteristics as the other ε analogs. ε Adenine derivatives can't be distingished by this matter. 3′,5′-cyclic εAMP displays an intense fluorescent emission (λ_{max} 413 nm) upon excitation at λ_{max} 314 nm. This allows ready detection at very low concentration in the range at 10 nM and the presence of the long wavelength absorption of 314 nm is important, because it permits excitation of the fluorophore without interference from other ultraviolet absorbing moieties in proteins and nucleic acids. In view of all these favorable fluorescence properties

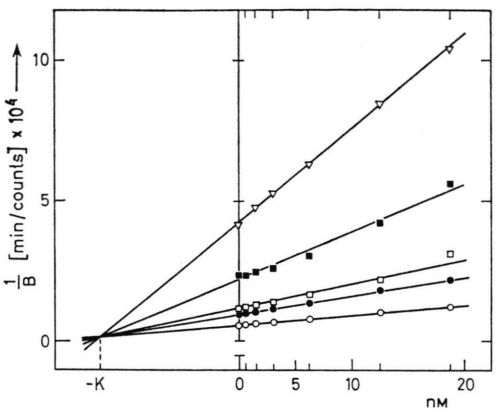


Fig. 4. "Single reciprocal plot"[4]. K_{diss}=12.5 nM. Concentrations of [³H]3′,5′-cyclic AMP are 0.83 nM (—○—○—), 1.66 nM (—●—●—), 3.32 nM (—□—□—), 4.15 nM (—■—■—), 8.3 nM (—▽—▽—). Concentrations of 3′,5′-cyclic εAMP see on x-axis.

we examined especially the extent of biological activity to which it can replace the natural cyclic nucleotide as enzymatic modifier. This was tested by binding to the protein kinase system of beef adrenal under conditions described earlier for other cyclic nucleotides[4].

Affinity of 3′,5′-cyclic εAMP to binding protein was found to lie in the same range as the constants published for 3′,5′-cyclic AMP[4,5]. From other investigations[4,6] it may be assumed that free-NH_2 at position 6 of purine ring is not decisively required for binding of 3′,5′-cyclic AMP. The result presented here gives additional support to this assumption, since this site is concealed in 3′,5′-cyclic εAMP.

The fluorescence properties of 3′,5′-cyclic εAMP in conjunction with full biological activity as reported here and elsewhere[3] make it to a valuable tool in the field of cyclic nucleotide research.

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