Raman Spectroscopy Study of Acid–Base and Structural Properties of 9-[2-(Phosphonomethoxy)ethyl]adenine in Aqueous Solutions

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ABSTRACT: The acid–base properties of the acyclic antiviral nucleotide analogue 9-[2-(phosphonomethoxy)ethyl] adenine (PMEA) in aqueous solutions are studied by means of Raman spectroscopy in a pH range of 1–11 and compared with the properties of its common adenosine monophosphate counterparts (5′-AMP, 3′-AMP, and 2′-AMP). Factor analysis is used to separate the spectra of pure ionic species ($PMEA^{2-}$, $HPMEA^{-}$, H_2PMEA, H_3PMEA^{+}) in order to determine their abundance, sites of protonation, and corresponding spectroscopic pK_a values. The characteristic Raman features of the neutral adenine moiety in $PMEA^{2-}$ and $HPMEA^{-}$ species resemble those of neutral adenine in the AMPs, whereas significant differences are observed between the Raman spectra of the N1-protonated adenine of the solute zwitterionic H_2PMEA and its N1-protonated AMP counterparts. On the contrary, the spectrum of crystalline H_2PMEA, adopting an "anti-like" conformation, is found to be similar to the N1-protonated AMPs in solution. To explain peculiar Raman features a "syn-like" conformation is suggested for N1-protonated PMEA species in aqueous solutions instead of an anti-like one adopted by H_2PMEA in crystals or by common AMPs in aqueous solutions. A physical mechanism of the anti-like to syn-like conformational transition of the solute PMEA that is due to adenine protonation and the flexibility of the (phosphonomethoxy)-ethyl group is proposed and discussed. © 2002 Wiley Periodicals, Inc. Biopolymers (Biospectroscopy) 67: 285–288, 2002

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INTRODUCTION

The acyclic nucleotide analogue 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) was synthesized by Holý and Rosenberg[1] and is representative of a class of agents with selective antiviral activity. PMEA (Fig. 1) was shown to be active against a broad range of herpes, hepadna-, and retroviruses, including hepatitis B and HIV.[2] Structure–activity studies[3] revealed that the antiviral activity of PMEA-like compounds is related to their molecular structure and might correlate with their preferred conformation in solution.[4] An evaluation of the physicochemical properties and conformational parameters of the solute PMEA

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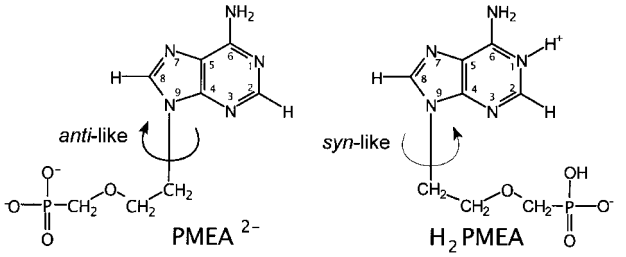

Figure 1. The molecular structures of dianionic $PMEA^{2-}$ and zwitterionic H_2PMEA shown in anti-like and syn-like conformations, respectively.

may be useful for understanding the mechanism of its action on a molecular level. The acid–base properties of PMEA and its N1, N3, and N7 deaza derivatives in aqueous solutions were already studied by ^{1}H-NMR spectroscopy and potentiometric pH titration[5] and compared with natural adenosine monophosphates (AMPs). It was shown that after protonation of the phosphonate residue, the most basic site in the fully deprotonated $PMEA^{2-}$ (pK_a ~6.9), the resulting $HPMEA^-$ species may be protonated at the N1 of the adenine residue to form zwitterionic H_2PMEA (pK_a ~4.16).[5] Analogously to AMPs, the monoprotonated $HPMEA^-$ was suggested to adopt an "anti-like" conformation,[5] which places the phosphonate group close to the H8 site of adenine. Certain indications of a small fraction adopting a "syn-like" form (phosphonate close to H2) were reported as well. A higher tendency toward the syn-like conformation was observed for some PMEA deaza derivatives.[5] Considering the high flexibility of the aliphatic chain of PMEA and the Coulombic attraction between the phosphonate group and the N1-protonated adenine, one can suggest that a syn-like conformation would be preferred for the H_2PMEA species. Nevertheless, in the crystalline state [monoclinic[6] (*m*) and orthorombic[7] (*o*)], the zwitterionic H_2PMEA was shown to exist in an anti-like conformation.

MATERIALS AND METHODS

The aqueous solutions (~25 m*M* in nucleotide, starting pH ~11, ionic strength adjusted by 0.1–4.0*M* NaCl) of PMEA, 2′-AMP, 3′-AMP, and 5′-AMP were gradually titrated using small volumes of an appropriate HCl solution (0.1–0.8*M*) to minimize dilution of the sample. The acid–base transitions between different ionic species were monitored by Raman spectroscopy (514.5 nm, 200 mW). The resulting spectra for each pH value were normalized by the use of an internal standard (1640 cm^{-1} band of H_2O) and corrected for the dilution effect and solvent contribution. Factor analysis and global fits to equilibrium equations were applied to obtain the spectra of the pure ionic species and to evaluate their relative fractions for each pH value.[8]

The Raman spectra of the polycrystalline H_2PMEA were excited at 488 nm. The normal modes, vibrational frequencies, and Raman scattering activities were calculated for the H_2PMEA *m*-[6] and *o*-form[7] crystal structures in the HF/6-31G* basis set using Gaussian 98. Raman differential cross sections were evaluated at ~300 K and an excitation wavelength (λ_{exc}) of 488 nm. The calculated spectra were scaled by 0.89 and plotted as a sum of Lorentzian curves with a half-width of 5 cm^{-1}.

RESULTS AND DISCUSSION

According to the potentiometric pH titrations,[5] four ionic species of PMEA were expected in the pH range of 1–11: dianionic $PMEA^{2-}$, monoanionic $HPMEA^-$, zwitterionic H_2PMEA, and monocationic H_3PMEA^+ [Eqs. (1)–(3)].

$$HPMEA^- \leftrightarrow PMEA^{2-} + H^+ \quad (1a)$$

$$K_{HPMEA} = [PMEA^{2-}][H^+]/[HPMEA] \quad (1b)$$

$$H_2PMEA^- \leftrightarrow HPMEA^- + H^+ \quad (2a)$$

$$K_{H_2PMEA} = [HPMEA][H^+]/[H_2PMEA] \quad (2b)$$

$$H_3PMEA^+ \leftrightarrow H_2PMEA + H^+ \quad (3a)$$

$$K_{H_3PMEA} = [H_2PMEA][H^+]/[H_3PMEA^+] \quad (3b)$$

Their characteristic Raman spectra are shown in Figure 2 along with typical spectra of the corresponding AMP ionic species. Despite some obvious differences issuing from the various molecular structures of 2′-AMP, 3′-AMP, and 5′-AMP, the Raman spectra of their particular ionic species exhibit virtually identical patterns, indicating identical sites of protonation and similar conformations; thus, 3′-AMP is shown as a representative case. Because the present study was focused on the acid–base transition between $PMEA^{2-}$ and $HPMEA^-$ [monoprotonation of the phosphonate group, Eq. (1)] and between $HPMEA^-$ and H_2PMEA [N1-protonation of adenine,

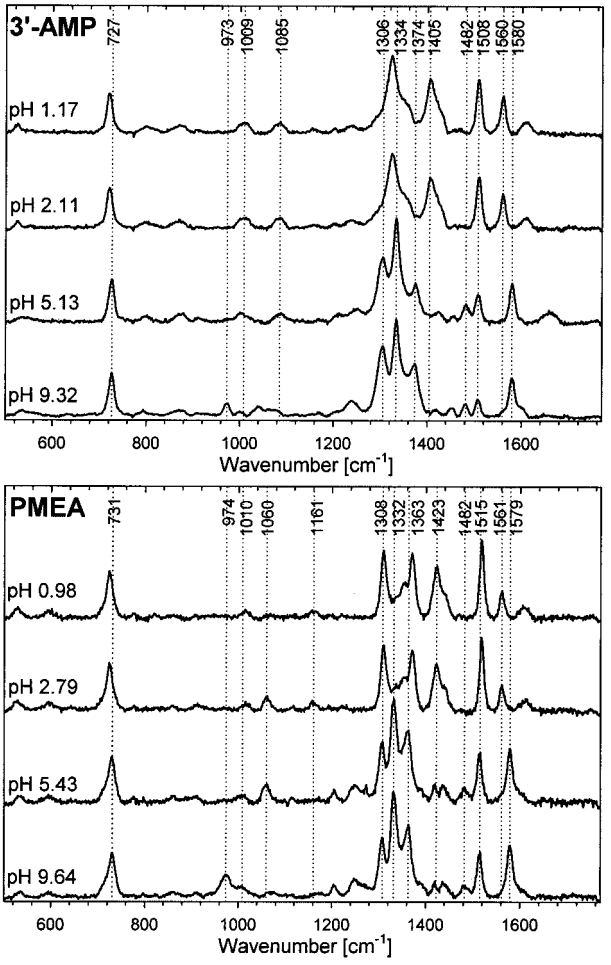

Figure 2. The measured Raman spectra of the PMEA solutions with prevailing ionic species of $PMEA^{2-}$ (pH 9.64), $HPMEA^{-}$ (pH 5.43), H_2PMEA (pH 2.79), and H_3PMEA^{+} (pH 0.98) in comparison with typical Raman spectra of their adenosine monophosphate counterparts (e.g., 3′-AMP): AMP^{2-} (pH 9.32), $HAMP^{-}$ (pH 5.13), and H_2AMP (pH 2.11 and 1.17).

Eq. (2)], only spectra from the pH 2.3–9.6 region were subjected to factor analysis.[8]

In comparing the spectra of $PMEA^{2-}$ and AMP^{2-} dianions obvious differences could be found in positions and relative intensities of particular bands of the neutral adenine residue (~650–780 and ~1200–1700 cm^{-1} regions). The most apparent is an unusually strong intensity of the 1515 cm^{-1} band of $PMEA^{2-}$ (upshifted for ~7 cm^{-1} with respect to AMP^{2-}) and a reversed intensity ratio of the 1308 and 1363 cm^{-1} bands (latter downshifted for ~11 cm^{-1}), whereas corresponding adenine bands of all AMP dianions studied exhibit virtually identical spectral features. Regardless of the apparent differences, the overall pattern of the $PMEA^{2-}$ spectrum matches well with its AMP^{2-} counterpart, indicating that $PMEA^{2-}$ is predominantly in an anti-like conformation,[5] similar to that of natural AMPs.

Monoprotonation of the phosphonate and phosphate groups [Eq. (1)] caused the indicative spectral changes in the 970–1085 and 970–1060 cm^{-1} regions, respectively. The corresponding pK_a 7.20 ± 0.16 of the PMEA determined by factor analysis is consistent with published results.[5] Because the factor analysis did not reveal any significant change of the $HPMEA^{-}$ adenine bands in the course of monoprotonation of the phosphonate group, it seems that $HPMEA^{-}$ generally preserves the same anti-like conformation as $PMEA^{2-}$.

As shown in Figure 2, the N1-protonation of the adenine residue [Eq. (2)] resulted in distinct changes of the H_2PMEA spectra. The overall character of the zwitterionic H_2PMEA spectrum (pH ~2.79) significantly differs from that of H_2AMP, mainly in the 1300–1450 cm^{-1} region. Instead of a single broad band centered at ~1324 cm^{-1} (3′-AMP) and observed in the spectra of all N1-protonated AMPs, two separate bands appeared at 1310 and 1372 cm^{-1} in the spectrum of H_2PMEA, querying N1 as a site of adenine protonation. Nevertheless, the appearance of the reliable N1-protonation Raman marker located at ~1561 cm^{-1} along with the ^{13}C-NMR results (data not shown) support N1-protonation, thus corroborating the interpretation based on the ^{1}H-NMR data.[5]

As shown in Figure 3, the Raman spectrum of the polycrystalline zwitterionic H_2PMEA exhibits an overall pattern of its adenine bands that is more similar to that of solute H_2AMP than to solute H_2PMEA. The most apparent is a single band at ~1341 cm^{-1}, corresponding better to the single ~1324 cm^{-1} band of N1-protonated H_2AMP than to features of the solute H_2PMEA. Because H_2PMEA in the crystalline state is known to be N1-protonated and to adopt an anti-like conformation,[6,7] the atypical spectral features of H_2PMEA in aqueous solution could be attributed to its syn-like conformation that is stabilized by an intramolecular Coulombic interaction between the N1-protonated adenine and the negatively charged monoprotonated phosphonate group, which is allowed by the high flexibility of the PME chain. The pK_a value of 4.24 ± 0.14 determined for the PMEA N1-protonation by factor analysis and global fits was found to match well with the published values.[5] Moreover, the

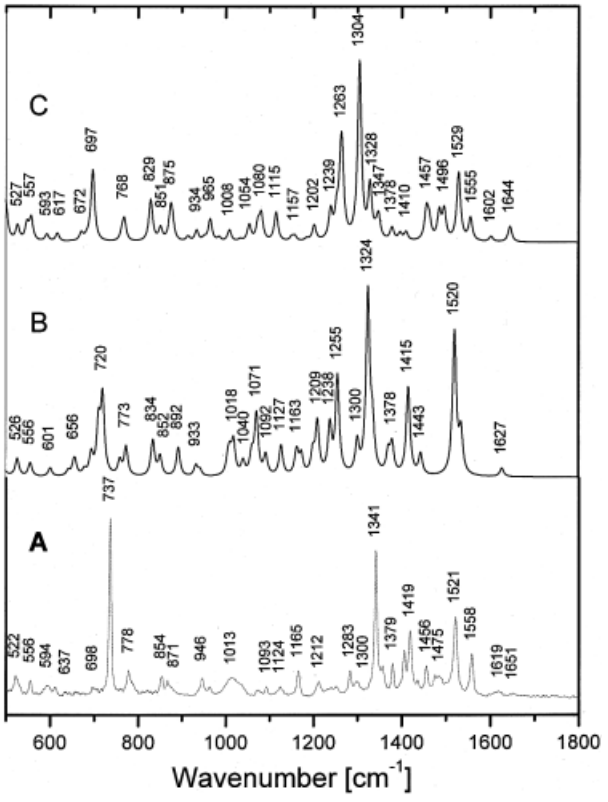

Figure 3. (A) The Raman spectrum of the PMEA polycrystalline powder sample compared with the *ab initio* calculated Raman spectra of the (B) monoclinic and (C) orthorhombic PMEA crystal structures.

slight but apparent changes in the phosphonate spectral region due to N1-protonation seems to support the above-discussed interpretation.

In accordance with Blindauer et al.,[5] a further site of PMEA protonation in a strongly acidic medium (pK_a ~1) was identified [Eq. (3)] on the phosphonate, as evidenced by the change of its 1060 cm^{-1} band (pH 0.98). Complete elimination of the negative charge of the phosphonate group did not lead to any detectable change of the adenine bands. Thus, H_3PMEA^+ seems to remain in the same conformation as adopted by H_2PMEA. Consequently, a putative syn-like conformation of H_2PMEA and H_3PMEA^+ is to be stabilized also by forces other than Coulombic attraction. This conclusion could be supported by titration experiments of PMEA under increasing ionic strengths. The increase of the ionic strength (data not shown) from 0.1 to 4*M* (NaCl) did not cause any significant change of the pK_a value of the N1-protonation, whereas the pK_a constant of the phosphonate group was decreased to 6.05 ± 0.15. Consequently, some hydrophobic interactions between the aliphatic chain and nucleobase seem to stabilize the syn-like conformation.

In a comparison of the *ab initio* calculated Raman spectra of H_2PMEA (Fig. 3) one could find that the *m* form fits the experimental spectrum better than the *o* form. Whereas the *m* form with a "glycosidic" torsion angle of 3.3° is representative of pure anti-like conformation,[6] the *o* form (torsion angle 33.4°), which has its phosphonate group considerably deviated from the adenine plane, acquires features of an intermediate between an anti-like and syn-like conformation.[7] A downshift of the 1324 cm^{-1} band (*m* form) to 1304 cm^{-1} (*o* form) could correlate with the increase of the glycosidic torsion angle.

The present results demonstrated that the replacement of the bulky deoxyribose-phosphate by the more flexible PME group is responsible for an atypical pH-induced conformational transition of the PMEA. Because of a higher conformational flexibility, H_2PMEA seems to adopt the syn-like conformation that was not observed for zwitterions of the natural AMPs. On the other hand, dianionic and monoanionic species of PMEA, prevailing in the physiological pH region, seem to exist predominantly in the anti-like conformation similar to that of AMPs.

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