Raman Depolarization Ratios in RNA and DNA are Sensitive for Sugar-Base Coupling

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SYNOPSIS

Polarized and depolarized Raman spectra are obtained for a number of synthetic polynucleotides containing adenine, uracil, and thymine bases. The depolarization ratios are determined by two methods: (1) by dividing the \(\perp\)-spectrum by the \(\parallel\)-spectrum and (2) after curve fitting. Overlapping bands, isotope splitting, reorientational broadening, and noncoincidence splitting affect the magnitude of the depolarization ratios over the band-width. For both Lorentz and Gauss curves these influences are simulated. A comparison of the Raman spectra of RNA and DNA molecules shows that the depolarization ratios for a number of similar base vibrations are different. The vibrational modes and the depolarization ratios of sugar vibrations are most sensitive to the structure of the polynucleotide. Base vibrations that have their potential energy distributed over base and sugar atoms also seem to be more sensitive to the structure. For instance the adenine vibrations at 1332 cm\(^{-1}\) and 1344 cm\(^{-1}\) in poly (dA) - poly (dT) have different depolarization ratios of, respectively, 0.36 and 0.28. This supports a previous assignment of the 1332 cm\(^{-1}\) band to a different sugar pucker (\(O_4\)-endo) than the \(C_2\)-endo corresponding with the 1344 cm\(^{-1}\) vibration. Assuming equal Raman scattering coefficients for this vibration, irrespective of the sugar pucker gives rise to the following ratio of the \(O_4\)-endo/\(C_2\)-endo in poly (dA) of 0.41, in poly (dA) - poly (dT) of 0.37, and in poly (dA-dT) - poly (dA-dT) of 0.41.

INTRODUCTION

The measurement of depolarization ratio \(\rho\) is useful in the assignment of vibrational bands in Raman spectra to particular motions of the nuclei. In the Raman spectra of polynucleotides most of the intense vibrations are polarized. As a consequence these vibrations have the same local symmetry as the molecular group in which they originate. Thus the ring vibrations occur in the planes of the bases and the phosphate groups involve symmetric stretching of PO bonds. Lord and Thomas reported \(\rho\) of a number of intense bands in spectra of RNA. This analysis enabled the assignment of bands to particular molecular groups constituting the polynucleotides. It was reported that the \(\rho\) of the uracil vibration at 1230 cm\(^{-1}\) was twice as large in the mononucleotide as in the single-stranded polynucleotide. No further sensitivity of the \(\rho\) was reported comparing mononucleotides and single-stranded polynucleotides.

Measurements on polynucleotides oriented both in a hydrodynamic flow and in single crystals showed that the off-diagonal elements of the various Raman tensors are much smaller than the diagonal elements. For the strong base vibrations, the \(\alpha_{xx}\) and \(\alpha_{yy}\) were found to be 10 times larger than the \(\alpha_{zz}\), where \(z\) is the direction of the helix axis. The dependence on the scattering geometry was large for the base vibrations and small for the sugar groups, suggesting that the latter were not well oriented. The observed \(\rho\) were used to identify different vibrational modes.

Recently the \(\rho\) of Raman bands in the spectra of mononucleotides, dissolved in protonated and deuterated water, were reported. The relative magni-
tude of the components of the Raman scattering tensor can be estimated, using a reasonable, but simplified, tensorial representation. For the base vibrations it was assumed that $\alpha_{xx}$ is perpendicular to the base plane. From this the relative magnitude for $\alpha_{xx}$ and $\alpha_{yz}$ could be estimated for some vibrations. Benevides et al. expanded this study to a double-helical guanine and cytosine containing Z-DNA and obtained relations for the magnitudes of the Raman tensor components, which helped the assignment of some sugar modes. The relative orientation of various residues in a sugar-phosphate-base conformer could be proposed on the basis of the measurement of the orientation of the Raman tensors.

The $\rho$ reported in the literature for Raman bands of (poly)nucleotides has been obtained by dividing peak amplitudes. However, overlapping bands, isotope splitting, reorientational broadening and noncoincidence splitting affect the magnitude of the $\rho$. In this article the spectral dependence of the $\rho$ is compared with the $\rho$ obtained after curve fitting both the $\perp$- and $\parallel$-Raman spectrum. The results obtained by the latter method are more accurate and are used to show that the $\rho$ of the base vibrations that have their potential energy distributed over the sugar group change with changes in the secondary structure.

**MATERIALS AND METHODS**

From Sigma we purchased the mononucleotides rAMP (Lot no. 90H7215), dAMP (lot no. 66C-7040) and rUMP (lot no. 48F05481). All polynucleotides were purchased from Pharmacia (poly[rA], lot no. AA4110P01; poly[dA], lot no. 301786011; poly[rU], lot no. AB4440P08; poly[rA]-poly[rU], lot no. QH82452101; poly[dA]-poly[dT], lot no. 2107860011; and poly[dA-dT]-poly[dA-dT], lot no. AE7870108). The nucleotides were dissolved in an aqueous buffer solution containing 93 mM NaCl, 10 mM Na$_2$HPO$_4$, 7 mM Na cacodylate, and the pH of the buffer was 7.2. The concentrations were 78 mM for rAMP and rUMP, and 20 mM for dAMP. The concentrations for the single-stranded polynucleotides were 78 mM bases for poly(rA) and poly(rU) and 14 mM bases for poly(dA). The double-stranded polynucleotides were dissolved in the concentrations of 14 mM base pairs for poly(rA)-poly(rU) and 10 mM base pairs for poly(dA-dT)-poly(dA-dT) and poly(dA)-poly(dT).

The Raman spectrometer consisted of an Ar$^+$ ion laser (Spectra-Physics 2025-03), a double monochromator (Jobin-Yvon HG2S), and a Hamamatsu R943-02 photomultiplier. A 90° scattering geometry was used. The scattered light passes through an analyzer (Spindler & Hoyer, 10K) with a suppression ratio of $10^{-4}$. The accuracy of the measurement of the $\rho$ is improved by quasisimultaneously measuring the $\parallel$- (vertically polarized excitation and vertically polarized scattered light) and $\perp$- (horizontally polarized excitation and vertically polarized scattered light) spectra. At each frequency, the vertical component of the signal was recorded with and without a $\lambda/2$ plate in the excitation path; hence corresponding spectral points in the $\parallel$- and $\perp$-spectra are obtained at exactly the same monochromator position. The $\rho$ of the 667 cm$^{-1}$ band in chloroform was determined to be 0.011. This is smaller than the value of 0.014 previously reported, indicating that the polarization purity both in excitation and emission is of good quality.

The slitwidths of the monochromator were set to 400 μm. The resolution of the setup was determined from the response to the Ar$^+$-ion laser emission at 514.5 nm and the He-Ne laser emission at 632.8 nm. The setup response was well described, with a Gaussian convolution with a bandwidth of 3 cm$^{-1}$. All the spectra were deconvoluted with the setup response.

The transmission and detection sensitivity of the setup was characterized with the emission of a tungsten lamp. The deviation of the response from the emission curve of the black-body radiator was used for intensity calibration. The excitation wavelength was 514.5 nm.

The spectra for rAMP, rUMP, poly(rA), and poly(rU) were recorded with 15 seconds per spectral point. The spectra of the double-stranded nucleotides were recorded with 55 seconds per spectral point.

The amplitude depolarization ratios are calculated as the quotient of the $\perp$- and $\parallel$-spectra, both corrected for buffer and residual background contributions. The error in this amplitude $\rho$ is dependent on the resultant Poisson noise in the spectra. This error has been calculated for two adenine vibrations in poly(dA): the almost completely polarized band at 727 cm$^{-1}$ and the partially depolarized band at 1344 cm$^{-1}$. The 727 cm$^{-1}$ adenine vibration is a polarized band in a spectral region with a large background from the buffer. This gives rise to a maximal error. The measurement error in amplitude $\rho$ has been calculated to be 5%. For a depolarized band ($\rho = 0.28$), such as the 1344 cm$^{-1}$ band in adenine, the error is calculated as 1%.
The RAMPAC program was used to perform the data analysis and the curve fitting. This program is described by de Mul and Greve. The measured spectra were corrected for buffer contributions. Spectra of double-stranded structures were also corrected for a small residual background on the low-wavenumber side.

RESULTS

The spectral dependence of the $\rho$ is obtained by dividing the experimental $\perp$- and $\parallel$-spectra and reading the $\rho$ at the frequency of the peak maximum. This will be called the amplitude $\rho$. The amplitude $\rho$ depends on the estimate for the zero-scattering level in both spectra. In Figures 1 and 2 the data are shown for ribonucleotides containing only adenine and uracil. Figure 3 shows the data for deoxyribonucleotides containing only adenine and thymine. Our amplitude $\rho$ are on the average 0.03 larger than the values of Ueda, which is possibly due to differences in this estimate of the background.

From the data of Figures 1 to 3, a quick overview of the changes can be obtained. Changes in the $\rho$ occur in the frequency region between 800 cm$^{-1}$.
Figure 4. The effects of (A) overlapping bands, (B) noncoincidence splitting, and (C) reorientational broadening on the amplitude depolarization ratio for Lorentz bands. (A) Band I: \( \rho = 0.5, \omega_{\text{max}} = 20 \text{ cm}^{-1}, \Delta \omega = 11 \text{ cm}^{-1} \); band II: \( \rho = 0.333, \omega_{\text{max}} = 30 \text{ cm}^{-1}, \Delta \omega = 9 \text{ cm}^{-1} \). (B) \( \rho = 0.5, \omega_{\text{max}}^{\perp} = 20 \text{ cm}^{-1}, \omega_{\text{max}}^{\parallel} = 21 \text{ cm}^{-1}, \Delta \omega = 11 \text{ cm}^{-1} \), (C) \( \rho = 0.5, \omega_{\text{max}} = 20 \text{ cm}^{-1}, \Delta \omega^{\perp} = 11 \text{ cm}^{-1}, \Delta \omega^{\parallel} = 15 \text{ cm}^{-1} \).

and 1200 cm\(^{-1}\). Mainly the sugar and phosphate groups contribute here. The spectra of the mononucleotides show the PO\(^3\)\(^-\) vibration at 980 cm\(^{-1}\), whereas the spectra of the polynucleotides have the PO\(^2\) vibration at 1100 cm\(^{-1}\).

Changes in \( \rho \) can quickly be obtained from these figures; however the spectral dependence of the \( \rho \) is influenced by the choice of the zero-scattering level, overlapping bands with different \( \rho \), reorientational broadening of the \( \perp \)-spectrum, and noncoincidence splitting.\(^{10,11}\) The accuracy with which \( \rho \) can be obtained depends on the intensity in both the \( \perp \) and the \( \parallel \)-spectrum. Most important is the appropriate choice of the background in the \( \perp \)-spectrum, being the weaker of the two. In a curve fit the background is a result of the overlap of the wings of the Lorentz curves. The physical level of zero scattering is not on the level of the minima in the spectrum, but lies below this level, thereby increasing the intensity in the spectra.

Apart from the estimate of the background all mentioned effects depend on the bandshape. In Figure 4A we show the effect of overlapping Lorentz bands with different \( \rho \). Isotope splitting is considered as a case of overlapping bands. The resulting amplitude \( \rho \) are a mix of the physical values and the physical \( \rho \) are not obtained at any frequency position. The resulting error depends predominantly on the band separation relative to the bandwidths. Figure 4B illustrates the effect of noncoincidence splitting. A realistic value of 1 cm\(^{-1}\) is chosen\(^{10,11}\) and the vibrational bandwidth is 11 cm\(^{-1}\). In Figure 4C the effect of a reorientational broadening of 4 cm\(^{-1}\) on a bandwidth of 11 cm\(^{-1}\) is shown for a Lorentz bandshape. The actual \( \rho \) is 0.5, but it is observed that the \( \rho \) varies appreciably over the bandwidth. In Figure 5A, 5B, and 5C the same cases are shown for Gauss bandshapes, again showing a complex frequency dependence of the \( \rho \). One observes that the \( \rho \) has significant dispersion over the bandwidth and, hence, that the value of the depolarization ratio cannot simply be obtained from the spectral dependence. In order to illustrate the effect in an experimental case, the \( \perp / \parallel \)-spectrum of CHCl\(_3\) (Figure 6) was simulated. Introducing the effects of reorientational broadening and neighbouring bands gives a proper reconstruction of the spectrum of the \( \rho \). The increase at lower frequencies and the decrease at the higher frequencies are caused by overlap of the two vibrational bands. The \( \rho \) of 0.008 at the peak maximum at 667 cm\(^{-1}\) is the result of reorientational broadening. The real value is 0.011. The decrease by reorientational broadening is partly compensated by an increase due to the overlap with the anisotropic band at 760 cm\(^{-1}\). The experimental amplitude \( \rho \) shows a dip at 650 cm\(^{-1}\), which is caused by a weak, polarized band at this frequency. It is also concluded from this experimental result that the actual \( \rho \) cannot simply be obtained from the spectral dependence of the \( \rho \); a curve-fit analysis is necessary to obtain physical \( \rho \) values.

The \( \rho \) based on the intensities from the curve fit has been obtained for the nucleotide vibrations. The 785-cm\(^{-1}\) uracil vibration has a \( \rho \) of 0.07, and no changes could be observed as a function of the secondary structure. The previously reported\(^3\) difference in \( \rho \) for the 1230 cm\(^{-1}\) between rUMP
and poly(rU) is not in agreement with our data. The \( \rho \) is 0.32 in both cases. In Figure 7A–F the \( \rho \) of the strong adenine base vibrations has been compared for different secondary structures. The \( \rho \) of the 727 cm\(^{-1}\) is small and no significant changes occur that are larger than the error in \( \rho \). The \( \rho \) of the band of adenine at 1378 cm\(^{-1}\) does not depend on the type of sugar in the nucleotide or on the conformation of the sugar group in the polynucleotide. The adenine band at 1305 cm\(^{-1}\) can be compared between rAMP, unstacked poly(rA), dAMP, and poly(dA). No differences are observed. In stacked poly(rA) this band is split in two components and in double-stranded structures overlap occurs with uracil or thymine modes.

On the other hand, some adenine base vibrations show different \( \rho \) in different structures. The 1480 cm\(^{-1}\) adenine vibration has a \( \rho \) of 0.18 in double-stranded structures and 0.09 in single-stranded structures and mononucleotides. The 1575 cm\(^{-1}\) has a \( \rho \) that is 0.1 lower in double-stranded RNA than in single-stranded RNA and RNA mononucleotides. This difference could not be observed in DNA. The \( \rho \) of the 1506-cm\(^{-1}\) adenine vibration changes with a change in order of the helical structure. In mononucleotides and unstacked poly(rA), the \( \rho \) is 0.1–0.13 lower than in stacked structures. The vibration at 1423 cm\(^{-1}\) is more intense in deoxyribonucleotides than in ribonucleotides. The depolarization ratio of this band is 0.5 in double-stranded and single-stranded polydeoxynucleotides and also in dAMP. In polyribonucleotides the depolarization ratio is approximately 0.2 and approximately 0.3 for rAMP, as can be observed from the amplitude data (Figures 1 and 3). The 1220-cm\(^{-1}\) vibration changes in \( \rho \) upon melting of single-stranded poly(rA). In stacked poly(rA) the \( \rho \) is 0.45, whereas in rAMP and unstacked poly(rA) it is 0.25. In poly(dA) the \( \rho \) is 0.24, and in dAMP it is

**Figure 5.** The effects of (A) overlapping bands, (B) noncoincidence splitting, and (C) reorientational broadening on the amplitude depolarization ratio for Gauss bands. (A) Band I: \( \rho = 0.5, \omega_{\max} = 20 \text{ cm}^{-1}, \Delta \omega = 11 \text{ cm}^{-1} \); band II: \( \rho = 0.333, \omega_{\max} = 30 \text{ cm}^{-1}, \Delta \omega = 9 \text{ cm}^{-1} \). (B) \( \rho = 0.5, \omega_{\max} = 20 \text{ cm}^{-1}, \omega_{\max}^{\perp} = 21 \text{ cm}^{-1}, \Delta \omega = 11 \text{ cm}^{-1} \). (C) \( \rho = 0.5, \omega_{\max} = 20 \text{ cm}^{-1}, \Delta \omega^{\parallel} = 11 \text{ cm}^{-1}, \Delta \omega^{\perp} = 15 \text{ cm}^{-1} \).

**Figure 6.** (A) Amplitude depolarization ratio for chloroform (\( \perp/\parallel \)). Band I: \( \rho = 0.011, \omega_{\max} = 667 \text{ cm}^{-1}, \Delta \omega^{\parallel} = 5 \text{ cm}^{-1}, \Delta \omega^{\perp} = 8.5 \text{ cm}^{-1} \); band II: \( \rho = 0.54, \omega_{\max} = 760 \text{ cm}^{-1}, \Delta \omega = 38 \text{ cm}^{-1} \). (B) Total Raman spectrum of chloroform (\( \parallel + \perp \)).
Figure 7. Comparison of the intensity depolarization ratios of adenine vibrations for different nucleotides.
from curve fit:

\[ p(1332 \text{ cm}^{-1}) = 0.36 \]

\[ p(1345 \text{ cm}^{-1}) = 0.28 \]

Figure 8. (A) Dependence of the depolarization ratio of the adenine vibration around 1336 cm\(^{-1}\) on the sugar pucker in poly(dA). Different sugar puckers shift the adenine vibration. An O\(_4\)-endo sugar pucker is characterized by an adenine vibration at 1332 cm\(^{-1}\) with a \( p \) of 0.36 and a C\(_2\)-endo sugar pucker by an adenine vibration at 1345 cm\(^{-1}\) with a \( p \) of 0.28. (B) Total Raman spectrum of poly(dA) (\( \parallel + \perp \)).

0.15. This band is however overlapped by another vibration at 1204 cm\(^{-1}\), which makes the fit error as large as 0.05. The difference between stacked poly(rA) and the other structures, however, is larger than this error and this difference is observable also from the amplitude \( p \) in Figures 1 and 3. In double-stranded structures, this vibration is obscured due to strong uracil and thymine vibrations. In Figure 8, the vibrational bands around 1336 cm\(^{-1}\) of poly(dA) are shown with the amplitude \( p \) spectrum. In DNA the 1336-cm\(^{-1}\) adenine vibration of RNA splits in two components, with frequencies of 1332 cm\(^{-1}\) and 1344 cm\(^{-1}\). It is observed from both the amplitude data and the curve-fit results that the \( p \) of these two vibrations are different.

**DISCUSSION**

In Raman spectra of polynucleotides many overlapping bands occur. Curve fitting these spectra will give rise to accurate band parameters if the bands are separated in the order of their bandwidths. The error in the \( p \), which is defined as the quotient of two intensities measurements, will be larger if the \( p \) is close to zero, due to the uncertainty in the intensity of the \( \perp \)-spectrum. The error is smaller than 25% for the bands with a \( p \) smaller than 0.1, for instance for the bands at 724 cm\(^{-1}\) and 1480 cm\(^{-1}\). Most of the bands in the Raman spectrum have a \( p \) much larger than 0.1. For these bands the error in the \( p \) is smaller than 10%, becoming 5% for intense depolarized bands. The depolarization ratio of the base vibrations is shown in Figure 7.

The sugar-phosphate vibrations have a low intensity and a large bandwidth. The observed differences in \( p \) are large enough to be observed with amplitude data. In Figure 9, the sugar-phosphate vibrations are compared for poly(rA) and poly(dA). The 1100 cm\(^{-1}\) PO\(_4\) vibration does not change in \( p \) with changes in the secondary structure. However, large differences (up to 100%) can be observed for the vibrations of the sugar-phosphate bonds. A mechanical coupling of the atomic motions in the base and the sugar has been shown to exist, leading to a distribution of the potential.

Figure 9. Sugar phosphate vibrations have a different depolarization ratio and frequency in poly(rA) and poly(dA). (A) Amplitude depolarization ratios (\( \perp / \parallel \)). (B) Total Raman spectra of poly(rA) and poly(dA) (\( \parallel + \perp \)).
energy of the vibrational mode. This may explain the observed changes in \( \rho \) for some base vibrations. Letellier et al.\(^1\) showed, with normal mode calculations, that the oscillator frequencies of a number of base vibrations are sensitive to the glycosidic bond angle. It was calculated that in the anti-conformation the frequencies of the 1423 cm\(^{-1}\) and 1480 cm\(^{-1}\) vibrations were most sensitive. The depolarization ratio of the band at 1480 cm\(^{-1}\) is sensitive to conformational changes (Figure 7), and in particular to double-stranded formation. The band at 1423 cm\(^{-1}\) in deoxyribonucleotides has been assigned as partially due to the 2'CH\(_2\) scissoring mode of the deoxyribo group.\(^1\) Also in ribonucleotides a band at this position is present with a reduced intensity. As the 2'CH\(_2\) group has been replaced by the 2'CHOH, it is assumed that this band originates in the base residue. We find, experimentally, that the depolarization ratio of this base vibration depends on the type of sugar molecule. We therefore propose that the depolarization ratios of some base vibrations are connected to changes in the structure of the sugar-base conformer.

The Raman spectra of poly (dA), poly (dA-dT), and poly (dA-dT)·poly (dA-dT) show a number of indications for structural inhomogeneity.\(^1\) In the spectra of all these molecules a double-band structure appears with the positions at 1332 cm\(^{-1}\) and 1344 cm\(^{-1}\). Comparison of the spectra of poly (rA) and poly (dA) shows that the sum of the total intensities (I\(_{1}\) + I\(_{2}\)) of the two components in poly (dA) and the total intensity of the band at 1336 cm\(^{-1}\) in poly (rA) are comparable within 5%. It is reasonable to assume that in poly (dA) a single band has split in two bands as a result of the coupling of the adenine base vibrations to more than one sugar pucker, rather than the appearance of a totally new vibration. With molecular dynamics simulations, Brahms et al.\(^1\) proposed a dynamic sugar-pucker structure, in which the sugar pucker of the adenine base is 71% of the time C\(_{2\text{-endo}}\) and 29% of the time O\(_{4\text{-endo}}\) for poly (dA)·poly (dT). The results for poly (dA-dT)·poly (dA-dT) were similar. Brahms et al. assigned the 1344 cm\(^{-1}\) vibration to adenine coupled to the C\(_{2\text{-endo}}\) sugar pucker and the 1332 cm\(^{-1}\) vibration to an adenine coupled to the O\(_{4\text{-endo}}\) sugar pucker. This last assignment of the 1332 cm\(^{-1}\) adenine vibration was made on the basis of the molecular dynamics results. In poly (rA) the 1336 cm\(^{-1}\) adenine vibration is coupled to a C\(_{2\text{-endo}}\) sugar pucker and the \( \rho \) is 0.30. The much larger \( \rho \) of 0.36 of the 1332 cm\(^{-1}\) adenine vibration in polydeoxyri-
than those obtained from the amplitude depolarization ratio.

It was found that the values of the \( \rho \) of the adenine vibrations at \(~ 1336 \text{ cm}^{-1} \), \(~ 1423 \text{ cm}^{-1} \), and \(~ 1480 \text{ cm}^{-1} \) are sensitive to changes in the secondary structure. It is proposed that observed changes are due to coupling to the sugar-phosphate group. In adenosine ribose phosphates a C3'-endo sugar pucker at \(~ 1336 \text{ cm}^{-1} \) is found to be accompanied by a \( \rho \) of 0.3, while in adenosine deoxyribose a C2'-endo sugar pucker at \(~ 1344 \text{ cm}^{-1} \) and band at \(~ 1332 \text{ cm}^{-1} \) give rise to \( \rho \) of 0.28 and 0.36, respectively. A previous study assigned the band at \(~ 1332 \text{ cm}^{-1} \) to adenine ribose phosphates, \(~ \text{C}3',\text{endo} \) sugar pucker at \(~ 1344 \text{ cm}^{-1} \) and band at \(~ 1332 \text{ cm}^{-1} \) give rise to \( \rho \) of 0.28 and 0.36, respectively. A previous study assigned the band at \(~ 1332 \text{ cm}^{-1} \) to adenine residues connected to sugar residues with an O4'-endo pucker. This supports the results from this study that the depolarization ratio of base vibrations may depend on the conformation of the sugar residue. A further comparison between the residence times obtained from molecular dynamics calculations and the Raman intensities \((I_{\perp} + I_{\parallel})\) give corresponding occurrences of different sugar puckers in poly(dA), poly(dA)-poly(dT), and poly(dA-dT). The \( O_4'\)-endo/C2'-endo ratios are 0.41, 0.37, and 0.41, respectively.

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