Estimation of Poly A Secondary Structure from Raman Scattering in acid aqueous solution.

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Summary

In the frequency region 600–1600 cm^{-1} the Raman spectra of acidic aqueous solutions of poly (rA) consist of several well-resolved lines. Four of these lines at 725, 1303, 1336 and 1508 cm^{-1} demonstrate the Raman hypochromic effect of poly (rA) at pH-values of 5.73 and 5.35 as a function of the temperature.

The results suggest that Raman intensity measurements are sensitive to order-disorder transitions of aqueous polynucleotides.

Introduction

Physico-chemical studies of solutions of polyriboadenylic acid (poly (rA)) have been carried out in many laboratories[1-3] and it has been shown that poly (rA) exhibits properties similar to those of undenatured nucleic acids[4]. The molecular properties of the acidic form of poly(rA) have been interpreted in terms of a double-stranded helical model. This polynucleotide can undergo a reversible helix-coil transition that has been investigated by several spectroscopic methods. The Raman spectra of the commonly occuring polynucleotides have recently been published[5,6]. Laser Raman spectroscopy has become sufficiently sensitive to yield potentially valuable structural information about poly(rA), particularly in aqueous solutions. Raman frequencies of the bases were first reported by Malt[7]. These were followed shortly by a more comprehensive study by Lord and Thomas[8], who were able to make some rather general assignments of the bands they observed. The most careful investigation has been published by Small and Peticolas[5].

In agreement with their assignments , we have chosen bands at 725, 1303 and 1508 cm^{-1} in the poly (rA) spectrum as ring vibrations. Furthermore, we would like to discuss the intensity of the 1336 cm^{-1} band which increases markedly with temperature. The increase of the intensity of the Raman bands as a function of temperature is compared with the increase of ultraviolet absorption intensities at 260 nm as a function of temperature. We conclude that there is an extremly similar behaviour which can be interpreted in terms of an order-disorder transition of the secondary structure of poly (rA) in acid aqueous solution.

Experimental

Materials

Poly (rA) potassium salt was purchased from Boehringer, Mannheim, and used without further purification. To obtain the final polymer concentration of 2% (w / w), poly (rA) was first dissolved in deionized water and acetic acid and sodium acetate were added until the Na^+ concentration reached 0.1 M. The desired pH values were obtained by adding concentrated acetic acid dropwise to the polymer solution. NaCl was used to maintain a Na^+ concentration of 0.15 M in all solutions.

Measurements

Raman spectra were recorded on a Coderg PHO Raman Spectrometer equipped with a CRL Model 53 Å argon ion laser[9].
The 4880 Å -exciting line yielded about 1.5 Watt at the sample. All spectra were recorded at least twice. The temperature of the thermostated cell holder was controlled similar to the arrangement described by Small and Peticolas[10]. The temperature of the probe was measured by placing a junction of a thermocouple into the cell. The peak heights of the bands at 725, 1303, 1336 and 1508 cm^{-1} were measured and normalized by comparing them to their height at 15°C. The normalized heights were plotted against the temperature. The acetate band at 925 cm^{-1} and the poly(rA) band at 1100 cm^{-1} [11] were used as reference lines.

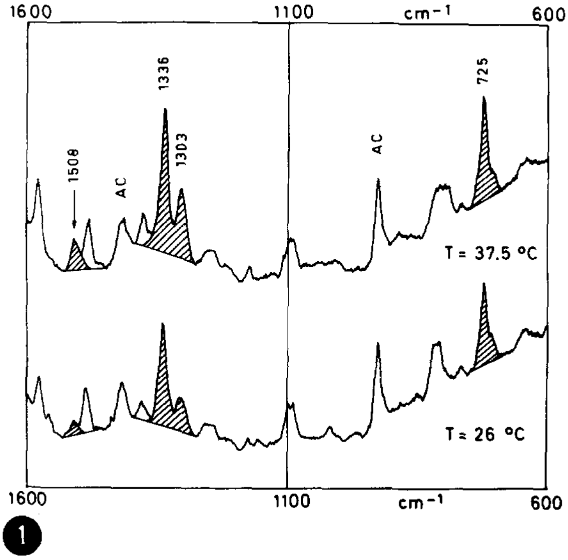
pH values were measured with a Radiometer (type 26, Kopenhagen) pH meter to 0.01 pH units. Calibration of the pH meter was performed with standard buffer solutions.

Results and Discussion

Figure 1 shows two original stripcharts of two Raman spectra of poly (rA) in aqueous solution at pH 5.73 recorded at 26°C and at 37,5°C. The bands shown here are at the same frequencies as those reported by Small and Peticolas for an aqueous solution of poly (rA) at neutral pH. One can clearly see, however, that the intensities have markedly increased in the spectrum at 37°C. The most pronounced increases are at 725, 1303, 1336 and 1508 cm^{-1}. The growth of intensity as a function of the temperature was observed in neutral solution by Small and Peticolas[5]. These authors created the term Ramanhypochromism for this experimental result as a paralell to the term "hyper-or hypochromic effect" in UV-spectra. The intensity of the 1336 cm^{-1} band was only slightly affected by increasing the temperature.

To make the Ramanhypochromic effect more pronounced, the normalized peak heights of the four lines were plotted against the temperature as is shown in Figure 2. Below 25°C the intensities of all bands are rather independent to temperature. In the temperature range from 25°C to 40°C, the intensities increase distinctly with temperature. This increase is due to the double helix coil transition of the polymer as was shown by independent physico-chemical methods such as ultraviolet spectroscopy or calorimetry[3,12]. Above 40°C the increase in the intensities is similar to that found in neutral poly (rA) solutions. This increase is due to the noncooperative unstacking of the bases in the partly ordered single-strands. The degree of stacking decreases with increasing temperature.

In Figure 3 the same plot is shown at a pH of 5.35 where the transition interval has shifted to 40-55°C, corresponding to a increased stabilization of the double helix of poly (rA) at the lower pH value. The general behaviour of the intensities as a function of the temperature is similar to



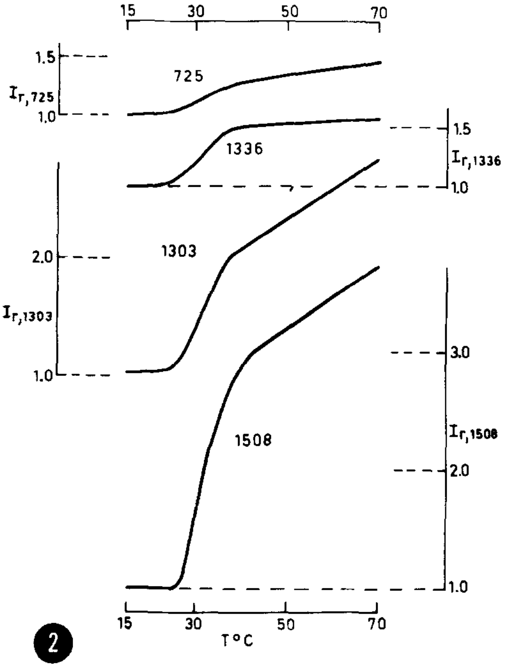


Fig. 1 Raman spectra of 2 % poly (rA) (w/w) at 26°C and 37,5°C, pH = 5.73

Fig. 2 Plot of the intensity of the bands at 725, 1303, 1336 and 1508 cm^{-1} in poly (rA) at pH 5.73 vs. temperature (ratio to intensity at 15°C)

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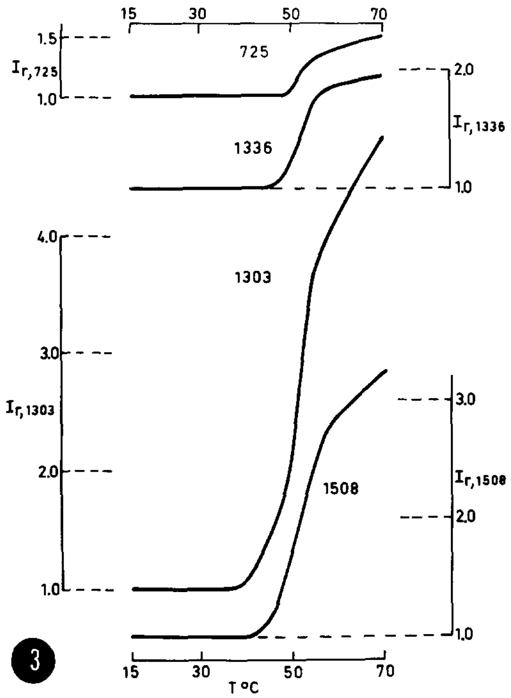
that shown in Figure 2. The increase is even more distinct, due to an increase of cooperativity of the bases. This result agrees well with UV-measurements.

One can see in Figures 2 and 3 that different lines show different relative changes of the intensities in the same temperature interval. This is shown more clearly in Figure 4 where the temperature dependence of two selected lines at 725 cm^{-1} and 1336 cm^{-1} is plotted at three different pH values, pH 7.0, pH 5.73 and pH 5.35.

We assume that the degree of structural ordering of the polymer is identical for all pH values at 70°C and therefore we normalized the peak heights to 70°C in this figure. Though, in detail, the variations in intensities of the lines (725 cm^{-1} and 1336 cm^{-1}) for different pH's, differ a lot one can find a similarity, viz. large intensity increases occur in the same



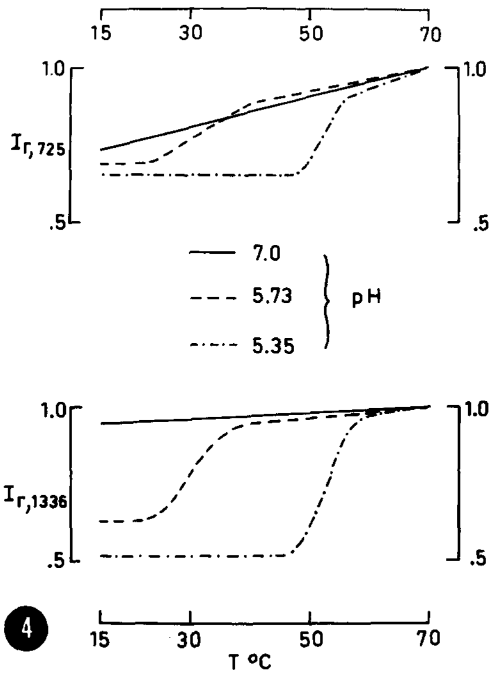


Fig. 3 Plot of the intensity of the bands at 725, 1303, 1336 and 1508 cm^{-1} in poly (rA) at pH 5.35 vs.temperature (ratio to intensity at 15^{o}C)

Fig. 4 Plot of the intensity of the bands at 725 and 1336 cm^{-1} in poly (rA) at pH's 7.0, 5.73 and 5.35 vs.temperature (ratio to intensity at 70^{o} C)

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temperature intervals for the acid pH-values.

The intensity of the 1336 cm^{-1} line, which is predominantly a C_5-N_7 stretching mode[13], is nearly temperature independent at pH 7.0. However, at the pH values of 5.73 and 5.35, this line shows a strong temperature dependence. From this we conclude that the intensity of this line (1336 cm^{-1}) has a much larger dependence on double helix coil transition than on the degree of ordering in the single strand. This suggests that the C_5-N_7 bond in the adenine molecule has different relative conformations in double helix and single strand.

In contrast to the behaviour of the 1336 cm^{-1} line, the intensity of the line at 725 cm^{-1} is temperature dependent at all three pH values. This means that the intensity of this line depends on the degree of ordering in the single strand as well as in the double helix.

These investigations show once more, that Raman spectroscopy may be useful tool for studying conformational changes in nucleic acids.

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References

1. J.R.Fresco and P.Doty, J.Am.Chem.Soc.79 (1957) 3928
2. DN.Holcomb and I.Tinoco Jr., Biopolymers 3 (1965) 121
3. H.Klump, T.Ackermann and E. Neumann, Biopolymers 7 (1969) 423
4. P. Doty, B.B.McGill and S. Rice, Proc.Nat.Acad.Sci.US.44 (1958)432
5. E.W.Small and W.L.Peticolas, Biopolymers 10 (1971) 69
6. N.N.Aylward and L.J.Koenig, Macromolecules 3 (1970) 590
7. R.A.Malt, Biochim.Biophys.Acta 120 (1966) 461
8. R.C. Lord and G.J. Thomas Jr., Spectrochim.Acta 23A (1967) 2551
9. E.D.Schmid, G.Berthold, H. Berthold, B.Brosa, Ber.Bunsenges. Phys.Chem. 75 (1971) 149
10. E.W.Small and W.L. Peticolas, Biopolymers 10 (1971) 1377
11. G.J. Thomas, M.C.Chen and K.A.Hartman ,Biochim.Biophys.Acta 324 (1973) 37
12. M.J.Massoulie, Compt.Rend 260 (1965) 5554
13. M.Tsuboi, private communication