STRUCTURAL INVESTIGATION OF POLY d(BrU-A) BY ULTRAVIOLET RESONANCE RAMAN SPECTROSCOPY*

L. Chinsky, P.Y. Turpin, M. Duquesne
Section de Physique et Chimie de l'Institut du Radium
Laboratoire associé au C.N.R.S. N°198
11 rue P. et M. Curie, 75231 Paris-Cedex 05, France
J. Brahms
Institut de Recherche en Biologie Moléculaire (C.N.R.S.)
Université Paris VII
2 Place Jussieu, 75221 Paris-Cedex 05, France

Received February 1,1977

SUMMARY

The resonance Raman spectra of a DNA containing bromodeoxyuridine (BrdUrd), the poly d(BrU-A), are reported, using U.V. laser as a source of excitation. The conformational change from the ordered, base paired form of poly d(BrU-A) (at 25°C) to the melted form at high temperature (63°C) is reflected in a pronounced hyperchromism of Raman bands at 1627 cm^{-1}, 1352 cm^{-1} and 1230 cm^{-1}. Particularly the band at 1627 cm^{-1} assigned to the vibrations of C4 carbonyl which is hydrogen bonded to adenine increases strongly its intensity upon melting. This represents a new approach for a detection of base unpairing and of modifications in geometry of selective molecules (BrdUrd) in a DNA chain in dilute solutions (10^{-4} M).

INTRODUCTION. Bromodeoxyuridine (BrdUrd), the analog of thymidine, has been widely used in biological investigation of DNA functions such as replication, transcription, cell differenciation and tumorigenicity in a variety of biological systems. BrdUrd when incorporated instead of thymidine in DNA permits almost normal cell division and metabolism in euracyotic cells (see ref.1 and references cited). In order to understand the molecular nature of these biological functions it is necessary to follow the chemical and structural changes of individual components and of selective chemical groups in DNA.

We present here a new approach for investigations of polydeoxynucleotide and DNA structure by the application of resonance

* Research supported by D.G.R.S.T.

ISSN 0006-291X

Raman spectroscopy. It is shown that using ultraviolet, pulsed laser beam as a source of excitation at the wavelength of 300 nm and a DNA which absorbs at the same wavelength, an enzymatically synthesized poly d(BrU-A), it is possible to observe selectively only one component BrdUrd. In contrast to the classical Raman spectroscopy, which uses light sources in the visible region and yields informations about the vibrations of all DNA components (2-4), one can expect that applying ultraviolet laser and very dilute concentration (10^{-4} M) only one absorbing selected chromophore, i.e. BrdUrd, will contribute to the resonance Raman spectrum (see ref.5 and 6). Other components of this DNA e.g., adenine, are not absorbing at 300 nm exciting frequency and are not contributing to an enhanced vibrational Raman spectrum.

MATERIAL AND METHODS.

Raman spectra were obtained with a Jarrel-Ash Model 400 double monochromator (slit width 6 cm^{-1}) modified with quartz optics for ultraviolet transmission. A Chromatix CMX4 pulsed tunable laser was used as ultraviolet light source at 300 nm wavelength. A digital data processing system was developped to integrate and to monitor the microsecond light pulses corresponding to the laser and to the Raman signals. The whole system would be described elsewhere.*

The poly d(BrU-A) was enzymatically synthesized using DNA-polymerase isolated from M. Luteus (7). BrdUTP was obtained from Terra Marina, poly d(BrU-A) was extensively deprotonized and dialyzed against 1 mM NaCl (pH 7).

RESULTS

The comparison of resonance Raman spectra of poly d(BrU-A) (Fig. 1.b) with that of BrdUrd (Fig. 1.a) shows clearly that all the observable bands are identical. The following bands are observed :

1) At about 1680 cm^{-1} and 1630 cm^{-1} the bands are assigned to carbonyl group stretching vibrations at position 2 and 4 respectively, on the basis of theoretical calculations of Tsuboi et al. (ref.2) of frequencies and normal mode of uracil and methyl uracil vibrations and of isotopic replacement by Miles (8).

*The electronics for the digital detection system was designed and developped in the laboratory (Laboratoire Curie - Institut du Radium) by L. Wolpert.

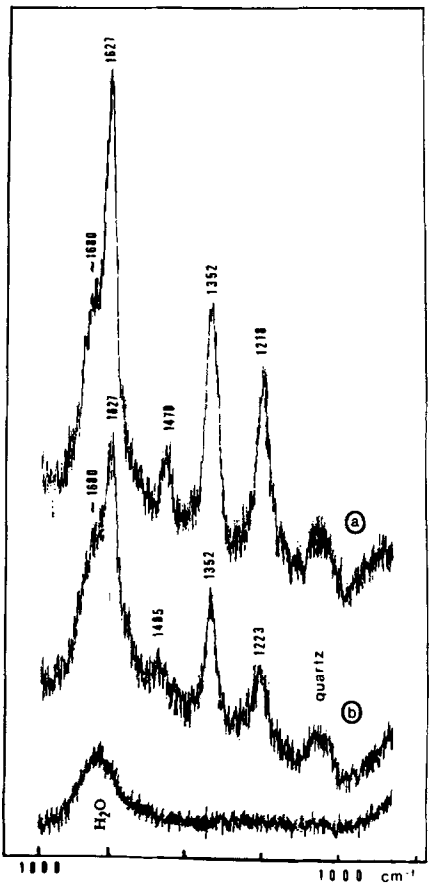

FIGURE 1

Comparison of resonance Raman spectra of 5-BrdUrd and poly d(BrU-A) measured under identical conditions (base concentration 1.3 x 10^{-4} M, excitation wavelength 300 nm); a) 5-BrdUrd - b) poly d(BrU-A). Each spectrum was reproduced up to 10 times.

2) The bands at 1420 cm^{-1} and 1230 cm^{-1} may be assigned at least partially to C5=C6 double bond stretching vibrations (see Tsuboi et al. ref.2) of BrdUrd.

Under the conditions of very dilute concentrations of nucleotides used in our experiments (10^{-4} M), it was necessary to take into account the possibility of contribution of water Raman bands, which was measured in control experiments (Fig. 1 bottom). It appears clearly

that owing to the enhancement of the chromophore (BrdUrd) Raman bands, water vibrational bands contribute relatively little to the resonance Raman spectrum, whereas in the classical Raman spectrum the region between 1600 cm^{-1} and 1700 cm^{-1} is obscured by the water contribution.

All these spectra were reproduced several times and no spectral changes were detected. Furthermore control experiments by a very sensitive differential method of thermal denaturation by absorbancy measurements at 260 nm of poly d(BrU-A) were performed before and after a series of resonance Raman measurements. The differential thermal denaturation profiles were identical. It can be thus concluded that there is no photochemical modification in the course of these spectral measurements. This is due to the features of ultraviolet, pulsed laser used, particularly of the relatively short duration of exciting pulse (few μ seconds) and of its relatively low average power (few milliwatts).

The resonance Raman spectra of poly d(BrU-A) were measured at low (25°C) and high (63°C) temperatures when the polymer is undergoing a conformational change from its ordered, base paired form to unordered, melted form. The intensity of the resonance Raman bands at 1230 cm^{-1}, 1420 cm^{-1} and at 1630 cm^{-1} increases by a factor of 2.2, 2 and 1.8 respectively. Comparison of poly d(BrU-A) Raman spectra measured at higher resolution in the region of 1500 cm^{-1} - 1800 cm^{-1} at low (25°C) and high (63°C) temperatures (Fig. 2) reveals that the band at 1627 cm^{-1} assigned to C4 carbonyl increases strongly its intensity, whereas the band at 1680 cm^{-1} assigned to C2 carbonyl vibrations shows essentially no changes. Thus the resonance Raman hypochromism is observed for C4 carbonyl group which is close to the C6=C5-Br part of the molecule, and is not observed for C2 carbonyl. The C4 carbonyl is hydrogen bonded, in the polymer, to NH_2 group of adenine and the changes in the intensity of the 1627 cm^{-1} band must be related to the changes of geometry when the "melting" of secondary structure occurs. No significant spectral

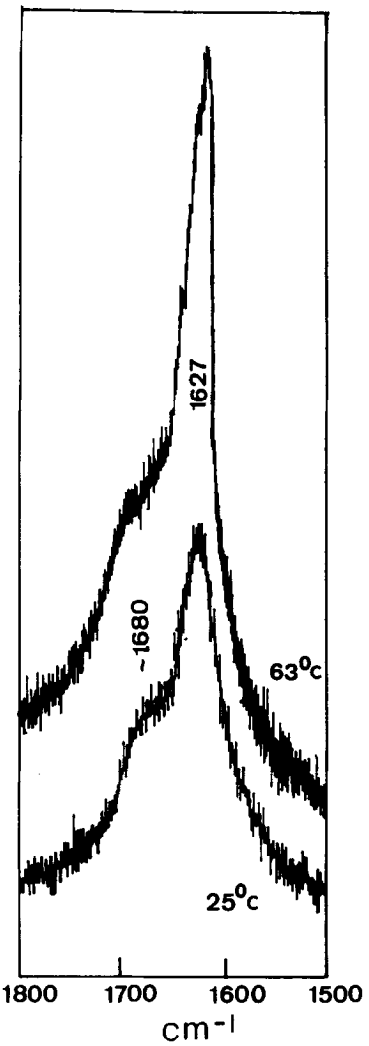

FIGURE 2

Resonance Raman spectra in the region of 1500 cm^{-1}-1800 cm^{-1} of poly d(BrU-A) at low and high temperatures (25°C and 63°C), i.e. in ordered and in melted, random coil form. Experimental conditions as in Figure 1.

shifts of the band position were observed. This fact was confirmed by measuring classical Raman spectra of poly d(BrU-A) at low (25°C) and high (63°C) temperatures using continuous argon laser beam (Coherent Radiation 52) as a source of excitation in the visible region, at the wavelength of 488 nm. Only relatively small increase in certain band intensities were observed upon "melting".

The absence of shifts in Raman bands position has been observed upon the melting of poly d(A-T) in contrast to the melting of poly A·poly U where a displacement of the 1681 cm^{-1} band has been reported (4,9). It seems that the substitution in the 5 position by methyl (in thymine) or by bromine (in BrdUrd) is responsible for these differences with uridine polymers.

In conclusion, the present work demonstrates the importance of resonance Raman hypochromism. These large changes in intensity of resonance Raman bands of 5-BrdUrd substituted polydeoxynucleotides should be a useful method for selective detection of changes in geometry in a small part of DNA molecule (or of chromatine). It will be also a useful approach for testing mechanism of replication, transcription and interaction of this DNA with specific proteins for which different hypothesis (of base unpairing, base tautomerization) were proposed (10-12).

REFERENCES

1. Silagi, S., Internat. Rev. of Cytology 45, 65-111 (1976).
2. Tsuboi, M., Takahashi, S. and Harada, I. in "Physico-chemical Properties of Nucleic Acids" (edited by Duchesne J. - Academic Press London-New York-) Vol.2 - p. 92-143 (1973).
3. Hartman, K.A., Lord, R.C. and Thomas, G.J. Jr. in "Physico-chemical Properties of Nucleic Acids" (edited by Duchesne J. - Academic Press London-New York) Vol.2, p. 1-90 (1973).
4. Small, E.W. and Peticolas, W.L., Biopolymers 10, 69-88 (1971).
5. Nishimura, Y., Hirakawa, A.Y., Tsuboi, M. and Nishimura, S. Nature 260, 173-174 (1976).
6. Chinsky, L., Turpin, P.Y., Duquesne, M. and Brahms, J., Biochem. Biophys. Res. Comm. 65, 1440-1446 (1975).
7. Harwood, J.J., Schendel, P.F. and Wells R.D., J. Biol. Chem. 245, 5614-5624 (1970).
8. Miles, H.T., Proc. Nat. Acad. Sci. U.S.A. 51, 1104-1109 (1964).
9. Small, E.W. and Peticolas, W.L., Biopolymers 10, 1377-1416 (1971).
10. Geffer, M.L., Ann. Rev. Biochemistry 44, 45-78 (1975).
11. Chamberlin, M.J., Ann. Rev. Biochemistry 43, 721-775 (1974).
12. Topal, M.D. and Fresco, J.R., Nature 263, 285-293 (1976).