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RAMAN SPECTROSCOPY STUDY OF SPECIFIC DNA BINDING BY BIS-NETROPSINS

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Introduction

The properties of DNA-binding drugs, with respect to possible structural modifications related to antitumoral activities have attracted the numerous studies [1]. Bis-netropsins (bis-Nts) are the members of the family of A-T specific antibiotics, which composed of fwo netropsin monomers linked by a flexible chain. The interest toward these drugs is because of their ability to recognize and bind to a specific feature of DNA. Thereby the activity of DNA-specific enzymes can be modulated. In the present work we carried out the Raman spectroscopy study of interaction of bis-Nt(—><—) and bis-Nt(<—>) with oligonucleotides (oligos) containing different AT-stretches.

Results and Discussion

The site-specific oligos for bis-Nt(<—>) (oligo 1) and bis-Nt(—><—) (oligo 2) and control nonspecific oligo (oligo 3) were chosen as DNA substrates. The Raman spectra of bis-Nt(<—>) and bis-Nt(—><—) in complex with the oligo 1, oligo 2 and oligo 3 as well as their spectra in solution are shown in Fig. 1 and Fig. 2. The most remarkable feature associated with bis-Nt binding is an upshift of the band near 1620 cm^{-1} assigned to amide *I* vibration [2]. In the spectra of the bound molecules this band upshifts in frequency ibout 10-20 cm^{-1} and grows in intensity. Moreover, an intense upshifted amide I band in observed only if specific binding takes place. The changes due to DNA binding are also found in the amide III region, which is an indicator of N-H bonding of bis-Nts [2]. Moreover, the changes induced by DNA binding are sequence-dependent. In addition, the bands near 1420 (P4) and 1450 cm^{-1} (P3) assigned to pyrrole vibrations change relative intensity upon binding to DNA. Unlike the amide vibrational modes, the $I_{P4/P3}$ ratio changes in a more complicated manner and is not strictly related to specificity of bis-Nt interaction.

These results provide evidence for several conformational features of bis-Nt/DNA complexes. One characteristic is changes in amide region vibrational modes. These

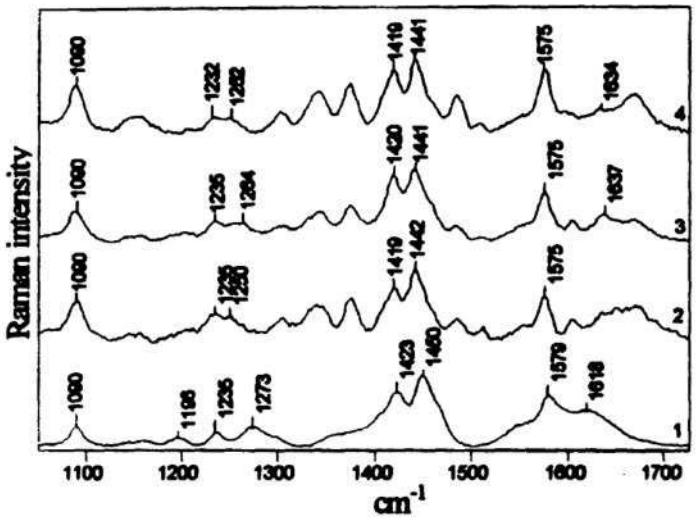

Fig. 1 Raman spectra in the region 1050-1750 cm^{-1} of: (1) bis-Nt(→←); (2) bis-Nt(→←)/oligo1; (3) bis-Nt(→←)/oligo2; (4) bis-Nt(→←)/oligo3. Molar drug/oligo ratio was 1:1.

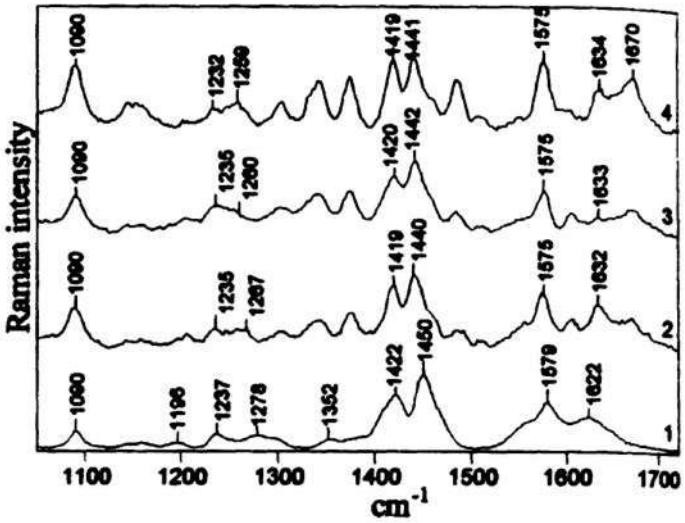

Fig. 2 Raman spectra in the region 1050-1750 cm^{-1} of: (1) bis-Nt(←→); (2) bis-Nt(←→)/oligo1; (3) bis-Nt(←→)/oligo2; (4) bis-Nt(←→)/oligo3. Molar drug/oligo ratio was 1:1.

should be consistent with H-bonding of peptide N-H groups to DNA. This conclusion is supported by the structural data on Nt/DNA complexes [3] and CD data on bis-Nt/DNA complexes [4]. Moreover, the degree of bis-Nt/DNA interaction is determined by a DNA sequence and can be followed by the position and intensity of the amide I band in the Raman spectra. A second characteristic observed is that the pyrrole ring vibrations are affected by ligand binding to DNA. Since bis-Nts are unlikely to intercalate this observation implies that direct interactions between other parts of the molecules and DNA induce stresses and alterations in the bond orbitals of the pyrrole rings.

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