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Vibrational mode analysis of guanine by neutron inelastic scattering

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Abstract. The low-temperature neutron inelastic spectrum of guanine has been measured. In order to assign the intense peaks observed in this spectrum, a normal mode analysis has been performed, using the Wilson GF-method. The theoretical treatment is based on a non-redundant set of internal coordinates, and a simplified valence force-field approximation. Only the fundamentals have been considered for simulating the internal vibrational mode spectrum. The calculations account for the spectral shape as well as the main observed peaks.

Key words: Neutron inelastic scattering – Vibrational modes – Raman and infrared spectroscopy – Nucleic acids – Normal mode analysis

Introduction

Neutron inelastic scattering (NIS) is a powerful method for detecting low-frequency molecular vibrational modes. NIS spectroscopy generally provides information complementary to that obtained from infrared absorption and Raman scattering. Moreover, there are several advantages in the use of NIS spectroscopy. First in neutron inelastic scattering processes, both energy and momentum transfer to the crystal phonons, are simultaneously of the required order of magnitude. Second, the absence of selection rules in the case of NIS spectroscopy allows one to detect a larger number of vibrational modes. In addition, neutrons are very sensitive to hydrogen motions because of its large incoherent cross section. The latter advantage is of considerable importance in the case of biological macromolecules which contain a great number of hydrogen atoms. In contrast, the vibrational motions in which the hydrogen atoms are involved do not provide enhanced Raman and infrared peaks.

The NIS spectra of nucleic acids have not yet been studied and here we have undertaken a preliminary study on the NIS spectra of nucleic acid bases. Knowledge of their vibrational modes can give important information on the hydrogen bonds between the complementary bases, and also on the stacking of the base planes, and also on the stacking of the base planes, when the nucleic acid double-helix is formed.

In the present work, results concerning guanine are reported. To carry out a discussion on the vibrational modes observed, the experimental results are accompanied by a normal coordinate analysis using the force-fields previously published (Majoube 1984; Letellier et al. 1987). Although, a similar expansion of the potential field in terms of internal coordinates is used, the out-of-plane mode force-constants have been refined in order to improve the agreement between experimental and simulated spectra. The reliability of the force-field used has been tested on the vibrational spectra obtained from deuterated derivatives of guanine as reported by IR or Raman spectroscopies (Majoube 1984).

Experimental

The NIS spectrum was obtained at 20 K with the time-focused crystal analyser TFXA, on the ISIS pulsed neutron source at the Rutherford Appleton Laboratory, UK (Penfold and Tomkinson 1986).

The guanine sample was a commercial product (Aldrich) and was contained in a thin-walled aluminium container. The NIS spectrum was recorded at low temperature to sharpen the fundamentals, by decreasing the effect of the Debye-waller factor (Jobic and Lauter 1988). The NIS spectrum of guanine as masured at 20 K is presented in Fig. 1 in the region below 250 mev (2000 cm^{-1}).

Calculation

Previously, a substantial study concerning Raman and Infrared spectra of guanine and its deuterated derivatives, has been reported by Majoube (1984). This work was

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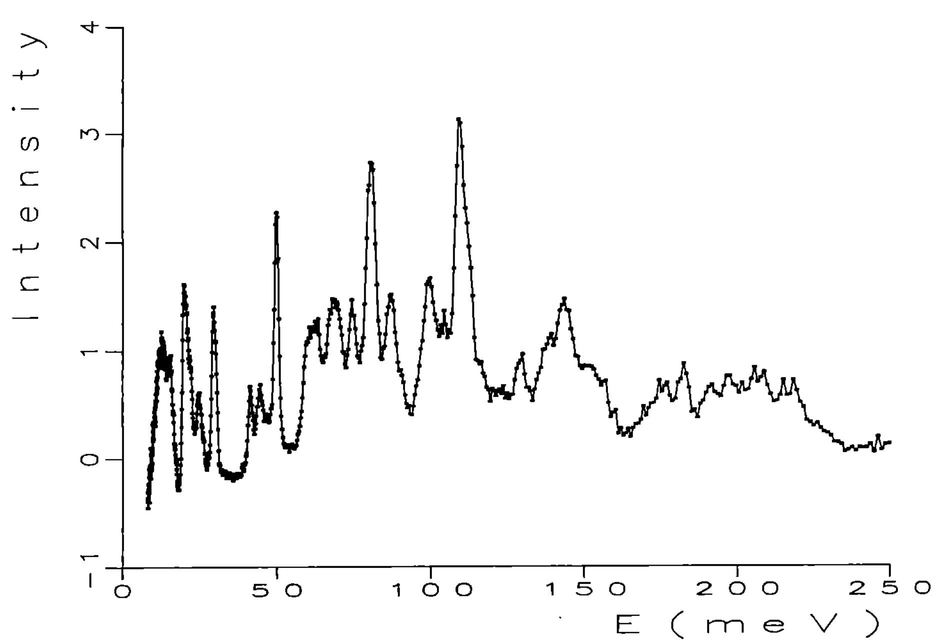

Fig. 1. NIS spectrum of guanine recorded at 20 K in the 0–250 mev spectral region (0–2000 cm^{-1}). The NIS intensity is reported in arbitrary units

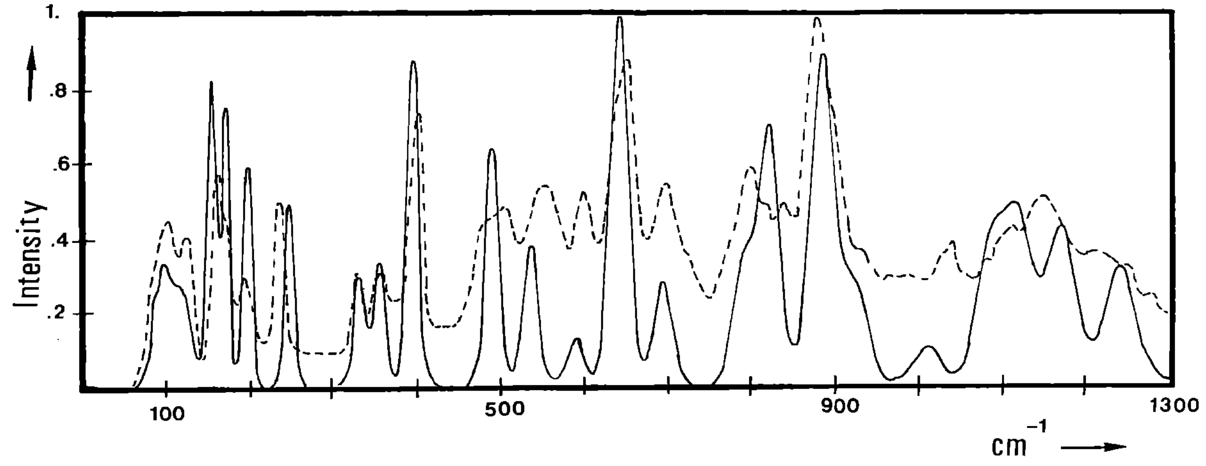

Fig. 2. Comparison between the experimental and simulated NIS spectra in the spectral region below 1 300 cm^{-1}. The NIS intensities are in arbitrary units. Only the vibrational fundamentals have been considered in the numerical simulation (see text). - - - - experimental NIS spectrum, —— simulated spectrum based on a first-order phonon-neutron interaction

Table 1. Comparison between the experimental and calculated vibrational mode wavenumbers (cm^{-1}) of guanine. The first number in parentheses represents the frequency-shift upon C8-deuteration, while the second corresponds to that observed upon deuteration of the nitrogens (G-d1 and G-d4 isotopic species, respectively. See text). The assignments are based on the potential energy distribution (PED), described in terms of internal coordinates. W: out-of-plane wagging, T: torsion mode

Vibrational spectroscopy (cm^{-1})	NIS (cm^{-1})	Calculated results	
		Wavenumber (cm^{-1})	Assignment (PED)
885 (+5, −)	928	900 (0, −195)	W (N9H) (22%); W (C2N2) (24%); T (N3C4) (13%); T (C2N3) (11%)
	872	881 (−151, +12)	W (C8H) (43%); W (N9H) (30%)
838 (−7, −)	828	820 (−13, −101)	W (N1H) (50%); T (N1C6) (18%); T (C2N1) (10%)
790 (+12, −)	800	789 (+51, +40)	W (N9H) (33%); W (C8H) (30%); T (N9C8) (13%); W (N1H) (11%)
654 (−6, −)	648	640 (0, −119)	W (NH2) (72%); W (N1H) (13%)
541 (−1, −13)	544	535 (−1, −59)	W (C6O6) (33%); T (C2N1) (21%); T (N1C6) (19%)
	500	488 (−40, −43)	T (N9C8) (35%); T (C8N7) (28%); W (N1H) (11%)
	400	394 (−12, −50)	W (C2N2) (29%); W (N1H) (26%)
	328	327 (0, −19)	W (C6O6) (31%); W (N1H) (26%); T (C5C6) (11%)
243 (+2, −3)	236	246 (0, −17)	T (C2N2) (25%); W (N9H) (12%)
204 (−2, −2)	196	196 (−5, −13)	T (N9C8) (24%); T (C4N9) (21%); W (N1H) (12%)
	188	170 (−3, −13)	T (C2N2) (24%); W (C2N2) (22%); W (N1H) (11%)
	160	153 (−3, −23)	T (C2N2) (29%); T (C2N3) (18%); T (N7C5) (16%)

based on a normal mode analysis of the planar vibrational modes (A' symmetry) of this molecule. Later, in order to complete the Majoube force field, a series of numerical calculations was reported on the out-of-plane modes (A'' symmetry) of guanine and its deuterated derivatives (Letellier et al. 1987). The present calculations have been performed in the light of the above-mentioned investigations.

To calculate the normal modes, the Wilson GF-method (Wilson et al. 1955) has been used. The redundancy between the internal coordinates has been entirely removed by the G-matrix diagonization procedure (Gusoni and Zerbi 1986). The simulation procedure for NIS spectra, is that explained in the past (Brunel et al. 1985). Only the fundamentals for phonon-neutron interaction have been taken into consideration. Normal mode frequencies, atomic displacements and NIS intensities for different vibrational modes have been calculated using a homemade program. Numerical computations have been performed on a SUN SPARC-SERVER 4/330, connected to a SECAPA 550 A graphic screen which allows the simulated spectrum, to be visualized and compared with the experimental one. The NIS spectral shape has been simulated by Gaussians. In order to take into account the instrumental resolution, the half-width of each Gaussian have been assumed to be a quadratic function of frequency. No calculation has been performed on the lattice modes which are observed in guanine in the spectral region below 150 cm^{-1} (Fig. 1). All the internal modes calculated in the present work are situated above 150 cm^{-1}. No interaction between the internal modes and those arising from the lattice vibrations has been considered in our simulation. However, the spectral shape in this region has been simulated by two Gaussians situated at 95 and 120 cm^{-1}, respectively.

Results and discussion

To begin our simulation, we introduced the force constants reported before by Majoube (1984) for the planar modes, and by Lettelier et al. (1987) for the out-of-plane modes.

Table 1 shows that most of the intense NIS peaks observed below 950 cm^{-1} arise from the out-of-plane vibrational modes (see also Fig. 1). For these modes, the initial force field could reproduce quite satisfactorily the vibrational wavenumbers. But unfortunately, a very poor agreement was obtained between the calculated and experimental intensities. In order to improve the agreement between the experimental and simulated spectra the initial force field was refined. The final values of the force constants are listed in Table 2. The comparison between the initial and final values (Table 2) shows that the interaction force constants are mainly altered by this refinement. This fact explains why the hydrogen atoms, responsible of the NIS intensities, are strongly coupled with the ring skeletal vibrational motions.

In Table 1 the calculated results obtained on the 13 out-of-plane modes of guanine and their assignments, have been given. In addition, to verify the reliability of the force-field obtained, the vibrational modes of two derivatives (G-d1: C8-deuterated; G-d4: N9, N2 and N1 deuterated) have been computed. The vibrational mode frequency shifts upon these selected deuterations, are reported in Table 1 and compared with those observed in the previous experimental investigations (Majoube 1984).

Table 2. Force-constant values (in mdyn A/rad^2) for the out-of-plane vibrational modes (A'' symmetry) of guanine. a Initial force-constants (Letellier et al. 1987). b Refined force-constants (present work). W: out-of-plane wagging coordinate; T: torsion-coordinate

Force-constants	(a)	(b)
Diagonal		
W (C8−H)	0.330	0.350
W (N9−H)	0.340	0.330
W (N1−H)	0.350	0.300
W (N−H2)	0.048	0.055
W (C=O)	0.450	0.334
W (C2−N2)	0.430	0.355
T (C−N) im.	0.490	0.520
T (C=N) im.	0.570	0.570
T (C=C)	0.520	0.520
T (C−N) pyr.	0.320	0.255
T (C=N) pyr.	0.629	0.629
T (C−C) pyr.	0.520	0.520
T (C2−N2)	0.080	0.100
Wagging−wagging		
W (C8−H), W (N9−H)	0.000	0.006
W (NH2), W (C2−N2)	0.010	0.028
Wagging−torsion		
W (N9−H), T (N9−C8)	−0.110	−0.137
W (N9−H), T (C4−N9)	0.110	0.137
W (C8−H), T (N9−C8)	0.070	0.153
W (C8−H), T (N7=C8)	−0.201	−0.153
W (C6=O6), T (N1−C6)	0.050	−0.056
W (C6=O6), T (C5−C6)	0.350	0.056
W (N1−H), T (N1−C6)	−0.140	−0.048
W (N1−H), T (N1−C2)	0.240	0.048
W (C2−N2), T (N1−C2)	0.020	0.056
W (C2−N2), T (C2=N3)	−0.037	−0.056
Torsion−torsion interactions		
T (C4−N9), T (N9−C8)	0.200	−0.029
T (N9−C8), T (C8=N7)	0.150	−0.029
T (C8=N7), T (N7−C5)	0.200	−0.029
T (N7−C5), T (C5=C4)	0.250	−0.029
T (C5=C4), T (C4−N9)	0.250	−0.029
T (C5=C4), T (C4−N3)	−0.300	−0.075
T (N2−C2), T (C2=N3)	−0.150	−0.075
T (C2=N3), T (N1−C2)	−0.100	−0.075
T (N1−C2), T (N1−C6)	−0.250	−0.075
T (N1−C6), T (C6−C5)	−0.250	−0.075
T (C6−C5), T (C5=C4)	−0.250	−0.075
T (C4−N9), T (C8=N7)	−0.050	0.000
T (N9−C8), T (N7−C5)	0.100	0.000
T (C8=N7), T (C4=C5)	0.050	0.000
T (N9−C8), T (C4=C5)	−0.050	0.000
T (C4−N9), T (N7−C5)	0.020	0.000
T (C5−C6), T (C2−N1)	−0.180	0.000
T (N1−C6), T (C2=N3)	−0.130	0.000
T (N1−C6), T (C4=C5)	−0.130	0.000
T (C2=N3), T (C4=C5)	−0.100	0.000
T (N3−C4), T (C5−C6)	−0.050	0.000
T (N3−C4), T (C2−N1)	−0.070	0.000

Finally, Fig. 2 shows the comparison between the simulated and experimental spectra in the region below 1 300 cm^{-1}. As can be seen, the spectral shape of the NIS spectrum has been satisfactorily simulated by the present calculations.

In conclusion, a preliminary simulation of guanine NIS spectrum based on a first-order treatment, allowed all of the intense peaks observed below 1 300 cm^{-1} to be assigned. The present study has allowed us to refine the out-of-plane mode force field, in order to have a better knowledge of the hydrogen motions and their coupling with the ring vibrations. It should be mentioned that this kind of analysis cannot be made on the basis of Raman or IR spectra, which essentially reveal only the heavy atom vibrations. NIS spectroscopy should thus be considered as a powerful probe for the study of the nature of the molecular vibrations, of nucleic acids, which contain a large number of hydrogens.

Of course, a more complete treatment needs the introduction of second and higher order spectra. The very low intensity and the bad resolution of the NIS bands situated above 1 300 cm^{-1}, do not allow a good assignment of the vibrational modes in this region. Our project in the near future, is to use the resonant Raman spectroscopy (RRS) which provides intense lines in the 1 800 – 1 300 cm^{-1} region. In a similar manner, the RRS spectral shape can be simulated (Peticolas et al. 1980). Finally, the RRS study will allow us to test the reliability of the planar mode force field.

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