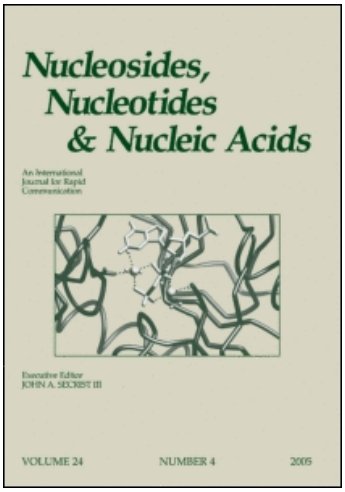

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CONSEQUENCES OF POLYMORPHISM OF 5′-GMP.Na_2 IN THE VIBRATIONAL SPECTRA OF METAL COMPLEXES AND ISOTOPIC DERIVATIVES

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ABSTRACT

The commercial mononucleotides are frequently used to obtain metal complexes and isotopic derivatives. Normally, the spectra of these new compounds are compared with the spectra of the commercial mononucleotides. Nevertheless, important variations in the vibrational spectra of the disodium 5′-guanosine monophosphate, 5′-GMP, have been observed in this work produced by submitting the commercial salt to the same general laboratory process that the obtained compounds, i.e., solving the commercial salt in water and subsequent recrystallization. These changes have been analyzed and interpreted. The variations are not significant in disodium 5′-cytosine monophosphate, 5′-CMP. It is important to take this information into account before carrying out vibrational studies with this type of molecules, since some bands attributed to isotopic substitutions or to the metal attack may be a result of manipulation of the nucleotide (solving and recrystallization) instead of the studied effect. Thus, before any work in which the nucleotide salt is manipulated (deuteration, synthesis of other isotopic derivatives or metal-nucleotide complexes), it should be noted that the process to which the sample is

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submitted on its own is enough to modify the vibrational spectrum. Then, attention should be paid to the changes observed in the vibrational spectra of recrystallized mononucleotides, since recrystallization may lead to a considerable phase change, and this can notably alter the vibrational spectra.

INTRODUCTION

The binding of metal ions to nucleic acids or to their components and the effect that metal ions have on the structure and conformation of these molecules, have been the object of many studies.[1–6] Thus, this work is included in a research field devoted to understand the way in which a metal (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cr^{3+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Al^{3+}, Ga^{3+}) interacts with nucleotides (5′-GMP and 5′-CMP), out of interest in these interactions themselves, and in order to widen the knowledge which may then be applied to the interactions between metals and DNA or RNA. These studies have been carried out in solid state (metal-nucleotide complexes), besides some systems in solution. The synthesis of the solid metal-nucleotide complexes was carried out in H_2O, the pH was adjusted at 7 with NaOH or HCl, and the temperature was between 35 y 40°C (approaching the physiological conditions). The disodium salts of each nucleotide were used.

In this context, it was necessary, firstly, to solve the following problem, which has not been considered previously: *Does the mere process to synthesize metal complexes affect the vibrational spectra of the nucleotide?* In this work it is shown that just the process of dissolving and recrystallizing the disodium 5′-guanosine monophosphate salt, 5′-GMP, modify the vibrational spectra of this compound. Such processes are used often to obtain metal complexes and isotopically substituted compounds. Therefore, it is very important to consider these results before carrying out vibrational studies with molecules of this type, not only for the study of metal-nucleotides interactions, but also in vibrational studies of isotopic substituted derivatives of nucleic acid components. Isotopic substitutions may give rise to false frequency shifts: this may be a result of manipulation of the nucleotide (dissolving and recrystallization) instead of the isotopic substitutions.

EXPERIMENTAL

The disodium salts of guanosine-5′-monophosphate (99.9%), 5′-GMP, and cytosine-5′-monophosphate (99%), 5′-CMP, were purchased from Sigma Chemical Co. These mononucleotides were solved in distilled and deionized water. The samples for the spectroscopic study were obtained after recrystallization both at pH 7 (adding HCl) and without any modification of the pH of the solution (pH $\sim$ 8).

The FT-IR spectra of these samples in KBr pellets were recorded under vacuum with an apodized resolution of 1.77 cm^{-1} (Res = 2 and Hamming apodizing function), coadding 1000 interferograms in a Bomem DA3.02 interferometer equipped with a DTGS detector and a KBr beamsplitter. The FT-Raman spectra were obtained using a Bomem DA3/DA8 accessory equipped with an InGaAs detector and a quartz beamsplitter. One thousand interferograms were coadded for these spectra. An apodized resolution of 3.54 cm^{-1} (Res = 4 and Bartlet apodizing function) was used. The exciting source was a Nd-YAG laser operating at 1064 nm

RESULTS AND DISCUSSION

Both, the IR and Raman spectra of 5′-GMP, show notable differences between the native nucleotide and the recrystallized compound (Fig. 1 and Fig. 2). The main differences are observed between 1800 and 1300 cm^{-1} (Fig. 1). In the Raman spectra a band at 1730 cm^{-1}appears clearly visible in recrystallized 5′-GMP, practically undetectable in the IR spectrum. The band of medium intensity at 1675 $cm^{-1}$of the native nucleotide practically disappears. These bands have been related to the νC=O stretch with contributions from hydrogen bonds or from interactions due to self-association processes of the guanine residues.[7–11] A medium band at 1643 cm^{-1} in the IR spectrum stands out which is not observed in the spectra of the recrystallized samples. In this part of the spectrum, it is well known that bands from vibrations of water molecules are expected. It is therefore likely that the difference observed is due to a different degree of hydration. Thus, the variations in the IR and Raman bands at high wavenumbers (Raman bands at 1730/1675cm^{-1}; IR bands at 1695/1680 cm^{-1}) may be related to the different content of crystallisation water, as this will have an influence on the network of hydrogen bonds that surrounds the guanine moiety, and on those in which the exocyclic groups of guanine are involved (carbonyl and amino groups). Bearing in mind the interpretations found in the literature and those given for protonated forms of this nucleotide,[12] we can propose that the Raman band of the recrystallized form at 1730 cm^{-1} is related to C=O groups with a smaller participation from hydrogen bonds. Similar effects on the C=O bond stretches have been observed in the vibrational modes of uracil base interacting with water molecules, by means of DFT calculations.[13]

Differences are also observed at lower wavenumbers. Thus, the Raman band of the native product at 1567 cm^{-1} appears in the recrystallized form at higher wavenumber, $\sim$1583 cm^{-1}. The IR band at 1573 cm^{-1} of the native nucleotide shifts to higher wavenumbers, 1580 cm^{-1} in the recrystallized product. Bands at 1579/1577 cm^{-1} (strong in IR and Raman) are observed in the spectra of guanine derivatives in solution. For 5′-GMP

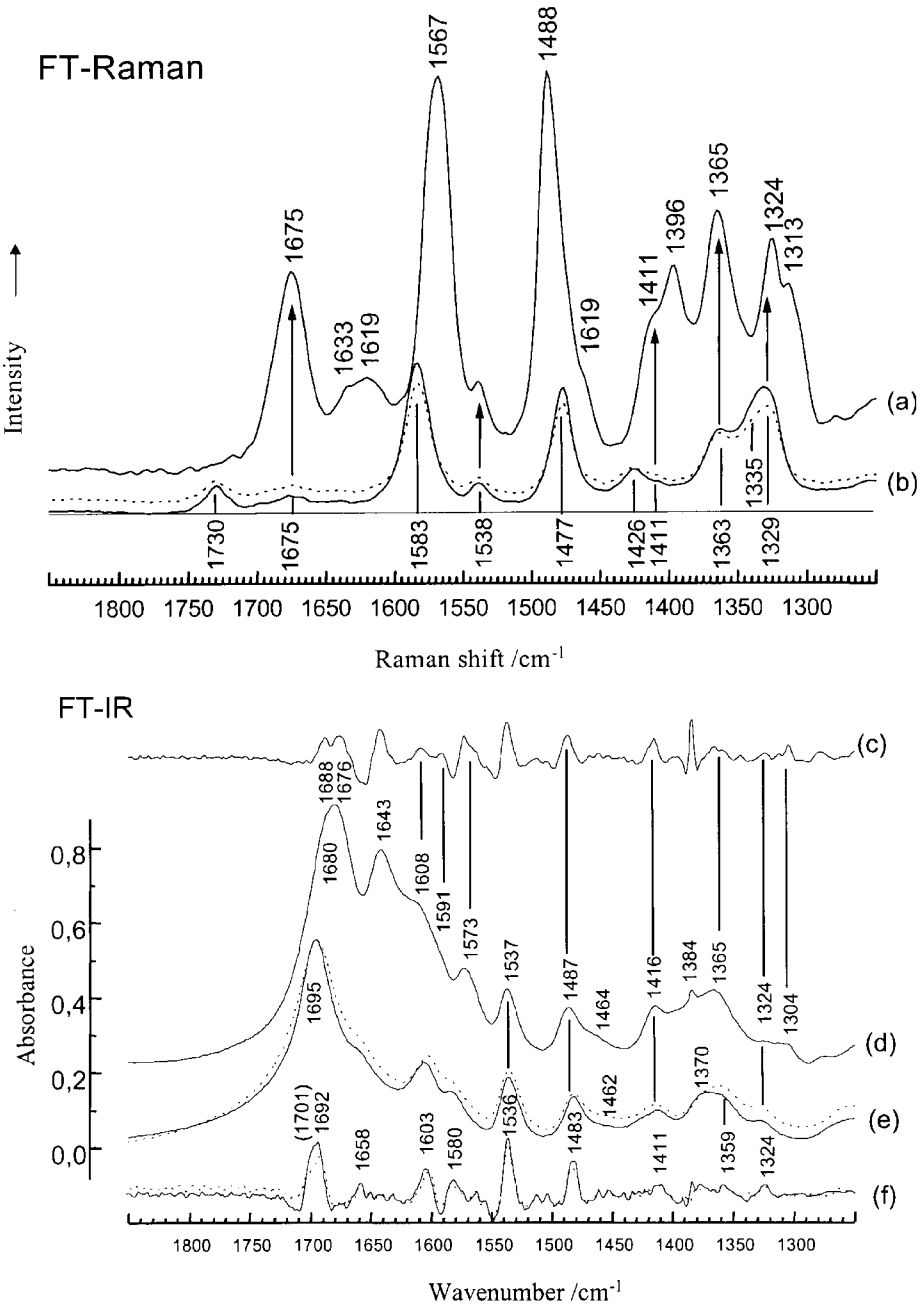

Figure 1. Variations of the IR and Raman spectra of 5′-GMP in solid state by recrystallization (1800–1300 cm^{-1}). FT-Raman: (a) commercial 5′-GMP; (b) recrystallized forms (full line = pH 8; dashed line = pH 7). FT-IR: (c) Second derivative with sign changed of the (d) spectrum ($-d^2A/d\nu^2$); (d) commercial 5′-GMP; (e) recrystallized forms (full line = pH 8; dashed line = pH 7); (f) Second derivative with sign changed of the (e) spectrum ($-d^2A/d\nu^2$).

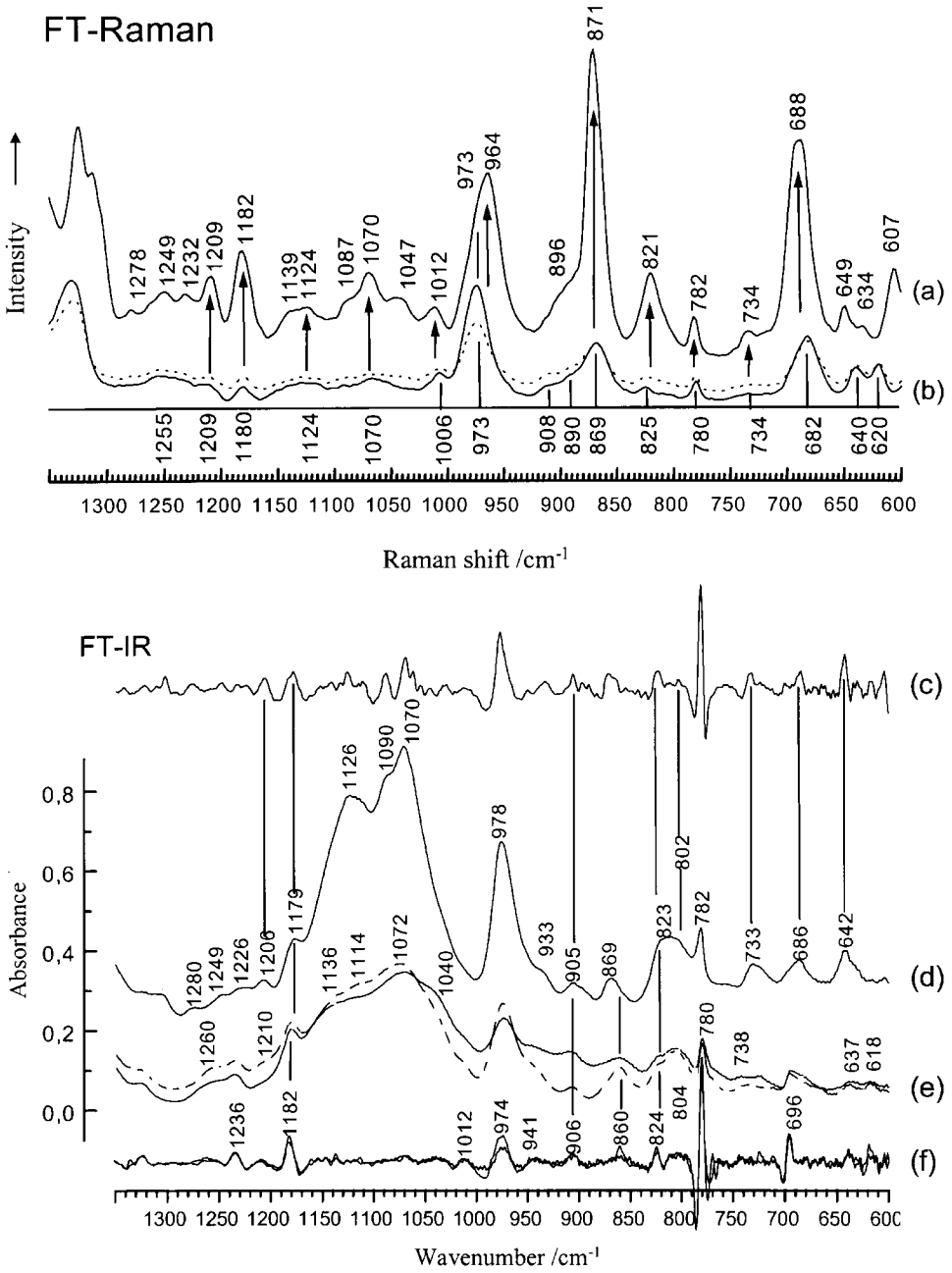

Figure 2. Variations of the IR and Raman spectra of 5′-GMP in solid state by recrystallization (1300–600 cm^{-1}). FT-Raman: (a) commercial 5′-GMP; (b) recrystallized forms (full line = pH 8; dashed line = pH 7). FT-IR: (c) Second derivative with sign changed of the (d) spectrum ($-d^2A/d\nu^2$); (d) commercial 5′-GMP; (e) recrystallized forms (full line = pH 8; dashed line = pH 7); (f) Second derivative with sign changed of the (e) spectrum ($-d^2A/d\nu^2$).

(neutral pH, 0.3 M and 25°C) these bands appear at 1578 cm^{-1} in H_2O and 1580 cm^{-1} in ^2H_2O,[14] with a shoulder at 1565 cm^{-1} (also in IR and Raman). The first band is assigned to the in-phase *v*C4=C5 + *v*C5-C6 mode, and the second to in-phase *v*C=O + *v*C5-C6 with 180° out-of-phase *v*C4=C5, and a possible contribution from C-N stretch, particularly the C2-N2 stretch.[8–10] As these modes give rise to bands with different components, changes of intensity in these overlapped components may induce the observed shift of the maxima. There is also a shift in the Raman band to 1488 cm^{-1} (native product), which appears at a lower wavenumber, 1477 cm^{-1}, in the recrystallized nucleotide. This band, which is strong in the IR and Raman spectrum, seems to be characteristic of guanine residues, being observed in vibrational spectra of guanine, guanosine and nucleic acids.[8–11, 15–17] Various assignments have been proposed such as that of stretches of ring double bonds,[16] δN1-H[9] or *v*N7=C8.[18,19] From the results obtained in studies of protonation and formation of metal complexes,[12] it can be affirmed that the region between 1480–1465 cm^{-1} includes vibrations in which N7, N9 and C8 participate. The mode that appears at ~1480 cm^{-1} being used in various studies as a marker of base stacking or metal ion bonding to the N7.[7] The frequency shift observed in the spectra may be explained in a similar way to the explanation given earlier for the 1567 cm^{-1} band; namely changes of the overlapped components of the two bands at ~1488 and 1477 cm^{-1}.

Between 1450 and 1300 cm^{-1} there are important variations, both in the IR spectrum and the Raman spectrum (Fig. 1). By means of isotopic substitutions[9,11] on guanine and its derivatives, it can be affirmed that the atoms involved in this localised vibration at ~1375 cm^{-1} (IR) for guanine[9] and 1368 cm^{-1} (Raman) for guanosine[11] are C8, N7 and N9, and H(C8). Specifically, it is proposed that the bands observed at 1375–1358 cm^{-1} (IR) and 1384–1354 cm^{-1} (Raman) arise from a combination of stretching and bending modes in the neighbourhood of C8.[10] On the other hand, the positions of the bands that appear between 1400–1300 cm^{-1} have been related to conformational parameters in the Raman spectrum of 5′-GMP.[14,20] Therefore, the large changes observed in this region could be due to a structural and conformational change of the 5′-GMP molecule. Nevertheless, to confirm this conformational change, the rest of the bands known as conformational markers must be checked. Thus, paying attention to the band that includes breathing vibrations of the guanine (682 cm^{-1} in the recrystallized form and at 688 cm^{-1} in the native sample, Fig. 2), it is observed that this variation does not correspond to that expected for ribose puckering changes. Then, along with the observation of the IR spectra, which do not indicate variations in the sugar folding either, lead us to think that there is not a conformational change of the ribose. Therefore, the differences in the 1400 to 1300 cm^{-1} region between the native and the recrystallized nucleotide should correspond to new vibrational modes arising from the

interactions to which the base is submitted in the native product. This agrees with that stated for the vibrations at a higher wavenumbers (1730–1650 cm^{-1}). At lower wavenumbers the differences are not relevant (Fig. 2). Contrary to the behaviour of 5′ -GMP, manipulation of the 5′-CMP disodium salt does not lead to significant changes in its spectrum.[12]

CONCLUSIONS

The changes observed in the spectra of 5′-GMP disodium salt are due to different crystallisation forms, to the number of water molecules that modify the hydrogen bonding network, to the polymorphism of the sample and, in general, to different types of interaction to which the guanine is submitted. Thus, in manipulated samples, such as deuterated 5′-GMP or any isotopically substituted or metal derivative, it should be taken into account that the mere process of dissolving and recrystallizing the nucleotide is sufficient to modify the vibrational spectrum, in addition to any expected isotopic or metal effects. Therefore, special attention should be paid to the changes observed in the vibrational spectra of recrystallized 5′-GMP, since the phase change due to recrystallization can notably alter the vibrational spectra. According to our experience, before any work in which the nucleotide salt is manipulated, it should be necessary to check the behaviour of the native mononucleotide under this treatment.

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