MOLSTR11872

ELSEVIER

Journal of Molecular Structure 000 (2001) 000–000

Journal of
MOLECULAR
STRUCTURE

www.elsevier.nl/locate/molstruc

Spectroscopic characterization of cyclic GMP in dimethylsulfoxide and water solutions

A. Tinti[a], V. Tugnoli[a,*], M.R. Tosi[b], D. Casarini[c]

[a]*Dipartimento di Biochimica, Università di Bologna, via Belmeloro 8/2, 40126, Bologna, Italy*
[b]*Istituto di Citomorfologia Normale e Patologica, CNR, via di Barbiano 1/10, 40129, Bologna, Italy*
[c]*Facoltà di Scienze, Università della Basilicata, via N. Sauro 85, 85100 Potenza, Italy*

Received 31 August 2000; revised 2 January 2001; accepted 2 January 2001

Abstract

We report the spectroscopic characterization of cGMP, which has an important biological role as a second messenger. NMR and Raman spectroscopies were used to characterize the cGMPH and cGMPNa structure in solution (water and dimethylsulfoxide). The spectroscopic results show that for cGMPH in dimethylsulfoxide the N7 position is free and the proton is localized on the phosphate group. By addition of 1 and 2 HCl equivalents to cGMPNa in the same solvent the first equivalent protonates the phosphate group while the second is localized on the N7 position of the guanine ring. On the contrary, for cGMPH in water the proton is mainly localized on the N7 guanine ring with an equilibrium depending on pH. Therefore, the solvent has a major effect in determining the protonation site in cGMPH. Solid state CP-MAS ^{13}C NMR spectrum of cGMPH suggests N7 protonation, phosphate group ionization and a sugar conformation different from that of the salt.

Keywords: cGMP; NMR; Raman; Solution structure

1. Introduction

A molecular structure — biological function relation has to exist for cyclic nucleotides (cGMP and cAMP) which are responsible for important biochemical events (i.e. second messengers) [1].

The aim of this paper was the characterization, by multinuclear magnetic resonance and Raman spectroscopies, of 3′-5′-cyclic guanosine monophosphate (cGMP), free acid (cGMPH) in comparison with its sodium salt (cGMPNa) in dimethylsulfoxide (DMSO) and aqueous solution. This was undertaken as an introduction to the study of the molecular interactions of cGMP with biological molecules like, for example, proteinkinase enzyme. In fact, the conformational profiles, the tautomeric characteristics and the different protonation degree may help to explain selectivity for binding activation of proteins by the cyclic nucleotides.

2. Experimental

cGMPH and cGMPNa were purchased from Sigma and used without further purification. The NMR spectra in D_2O and DMSO-d_6 solutions were recorded with a Bruker ACF 250 spectrometer using a multinuclear probe. ^1H chemical shifts of DMSO-d_6

* Corresponding author. Tel.: +39 51 209 4280; fax: +39 51 243 119.
E-mail address: matilde@ciam.unibo.it (V. Tugnoli).

0022-2860/01/$ - see front matter
PII: S0022-2860(01)00501-4

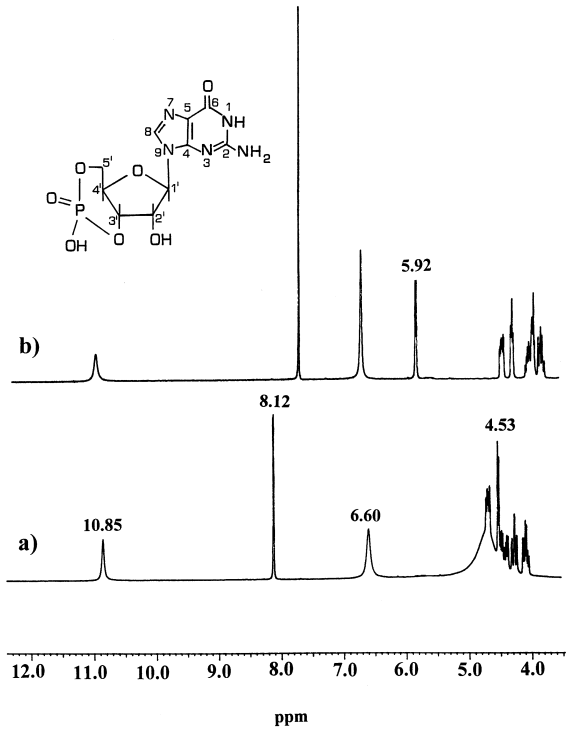

Fig. 1. ^{1}H NMR spectra of (a) cGMPH and (b) cGMPNa in DMSO-d_6.

solutions were relative to tetramethylsilane (TMS); ^{1}H chemical shifts of D_2O solutions were relative to trimethylsylil tetradeutero sodium propionate (TSP); ^{13}C chemical shifts were relative to TMS, while ^{31}P chemical shifts were measured with respect to 85% H_3PO_4 in D_2O. Experimental conditions were previously reported [2]. The Raman spectra were recorded with a Jasco R1100 with the 488 nm excitation wavelength at 2 cm^{-1} resolution. The solution concentrations were 0.05–0.1 M for all the measurements.

^{13}C solid state CP-MAS spectra were obtained using a Bruker CXP 300 spectrometer. The chemical shifts were reported with respect to TMS following the experimental conditions [3].

3. Results

Fig. 1 reports the ^{1}H NMR spectra between 3.5 and 12.0 ppm of cGMPH (a) and cGMPNa (b) in DMSO-d_6 solutions. Spectrum (a) is characterized by the following resonances: a singlet at 10.85 ppm, assigned to N1H, a sharp and intense singlet at 8.12 ppm, assigned to H8, a broad singlet at 6.60 ppm, arising from the NH_2 group of guanine moiety. The same resonances are present in spectrum (b) (cGMPNa); the singlet at 11.04 ppm appear broader and the strong singlet assigned to H8 is shifted by 0.3 ppm. Finally, the NH_2 singlet is slightly shifted and narrowed. The remaining resonances between 3.5 and 6 ppm are assigned to the ribose ring. In particular, the doublet at 5.92 ppm, assigned to H1′ in spectrum (b), was absent in spectrum (a). We tentatively identified this resonance in the doublet at 4.53 ppm.

Table 1 presents the ^{13}C NMR spectra of cGMPH

Table 1
^{13}C NMR chemical shifts of cGMPH and cGMPNa in various environments and in the solid state (* denotes the chemical shifts of the ribose ring for Guo and GuoH that are not reported because these compounds are not cyclic phosphates)

	C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′
cGMPH in DMSO-d_6	153.8	150.6	116.4	156.6	135.8	91.2	71.5	70.7	77.8	67.9
cGMPNa in DMSO-d_6	153.9	150.8	116.5	156.9	135.6	90.9	72.3	72.1	77.3	65.7
cGMPNa in DMSO-d_6 + 1 eq. HCl	154.0	150.7	116.4	156.6	135.9	90.9	72.2	72.0	77.3	65.8
cGMPNa in DMSO-d_6 + 2 eq. HCl	155.1	149.6	111.6	154.6	135.9	91.7	71.5	70.9	77.2	67.9
cGMPH in D_2O	152.2	147.3	108.6	153.8	134.3	90.1	69.1	69.3	74.4	64.6
cGMPNa in D_2O	151.1	148.3	113.8	156.1	135.4	89.4	69.5	69.2	74.2	64.5
Solid cGMPH	151.2	151.1	107.9	156.1	132.7	90.3	78.5	72.7	72.7	69.1
Solid cGMPNa	153.4	151.2	116.0	158.5	144.0	95.5	76.9	72.6	72.6	67.4
GuoH* in DMSO-d_6	155.8	150.0	109.5	154.4	135.6	–	–	–	–	–
Guo* in DMSO-d_6	153.6	151.3	116.6	156.8	135.7	–	–	–	–	–

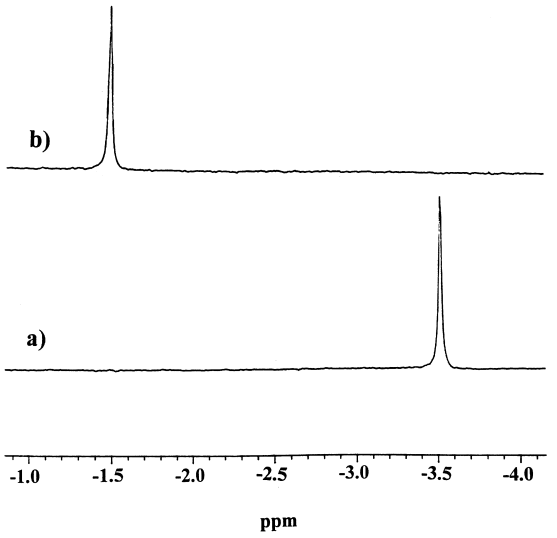

Fig. 2. ^{31}P NMR spectra of (a) cGMPH and (b) cGMPNa in DMSO-d_6.

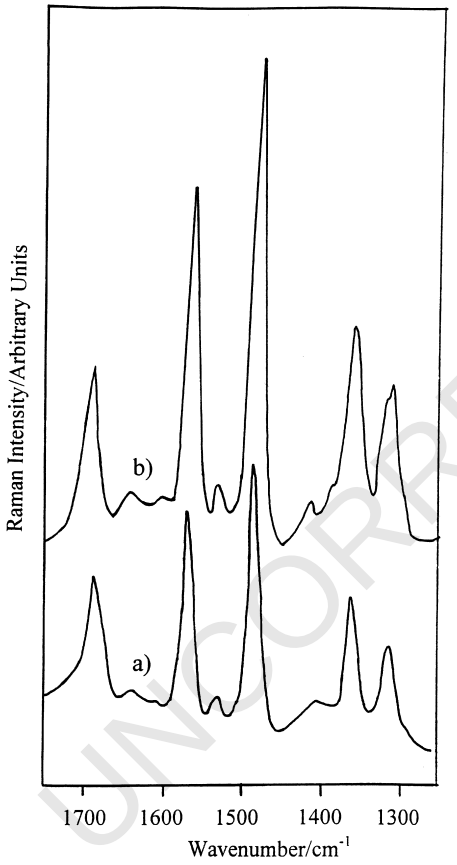

Fig. 3. Raman spectra of (a) cGMPH and (b) cGMPNa in DMSO-d_6.

and cGMPNa in DMSO-d_6 solution. The assignments of the guanine carbons were made on the basis of previously reported data for guanosine (Guo) in DMSO-d_6 [4]. The assignments of the ribose carbons have been obtained on the basis of their C–P coupling constants and are in agreement with those for cGMPNa in water [5]. cGMPH and cGMPNa are characterized by resonances with the same chemical shift values as the guanine residues, while the ribose carbons show significant differences, especially as regards C3′ and C5′.

Fig. 2 shows the ^{31}P NMR spectra of cGMPH (a) and its sodium salt (b) in DMSO-d_6 solution: two resonances are present at −3.5 and −1.5 ppm, respectively.

Fig. 3 shows the Raman spectra of cGMPH (a) and cGMPNa (b) in DMSO-d_6 solution in the range 1250–1750 cm^{-1}. The spectra are coincident as regards the frequency and relative intensity of the bands observed: they are at 1320, 1364, 1490, 1570 and 1697 cm^{-1}. The band at 1490 cm^{-1} is assigned to the ν_{10} guanine ring stretching mode [6] and is known to decrease in intensity when the N7 of the guanine ring interacts with electrophilic species (H^+, $-CH_3$, metal ions, etc.) [7]and the 1570 cm^{-1} band is assigned to ν_{11}.

Further measurements were performed adding to a DMSO-d_6 solution of cGMPNa 1 and 2 equivalents of HCl. The addition of the first equivalent showed a shift of the ^{31}P resonance from −1.5 to −3.5 ppm while the values of guaninic ring carbons, including C5, were unchanged. The second HCl equivalent caused a shift of the guanine ring resonances, including C5 which went from 116.4 to 111.6 ppm and the other carbons move upfield or downfield with the same trend observed for Guo and protonated guanosine (GuoH) in DMSO-d_6 [4]. Moreover, C3′ and C5′ of the ribose residue are also affected (see Table 1).

Unlike the DMSO-d_6 solutions, the resonances relative to guanine ring moiety differed in the ^{13}C NMR spectra of cGMPH and cGMPNa in D_2O (see Table 1) and showed the same trend as Guo and GuoH in DMSO-d_6. In particular, the C5 resonance was separated by 5.2 ppm for the two compounds. The ^{31}P spectra of cGMPH and cGMPNa in D_2O also behaved differently from those in DMSO-d_6 solutions: in water they showed the same chemical shift value

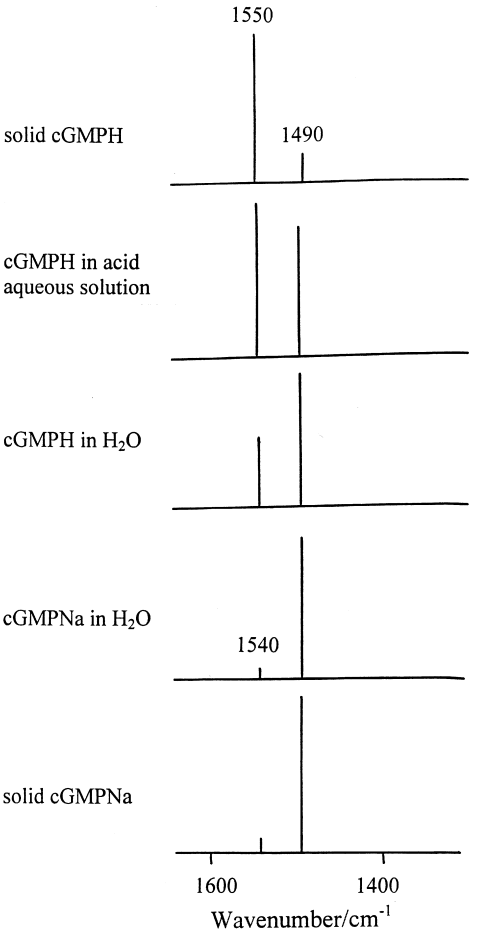

Fig. 4. Relative intensities of the Raman 1550 and 1490 cm^{-1} bands.

(−1.7 ppm) as the phosphate group resonance (spectra not reported).

The relative intensities of the Raman bands at 1490 and 1550 cm^{-1} of cGMPH and cGMPNa both solid and in aqueous solution are reported in Fig. 4. The relative intensities of these bands for cGMPNa in H_2O are similar to those of the same solid compound (1490 cm^{-1} very strong and 1540 cm^{-1} very weak). On the contrary, in the spectrum of cGMPH aqueous solution, the relative intensity of the 1550 cm^{-1} band increases but not as in the spectrum of the solid sample and the 1490 cm^{-1} intensity decreases slightly as regards the salt and the two intensities can be compared.

Fig. 5 presents the solid state CP-MAS ^{13}C NMR

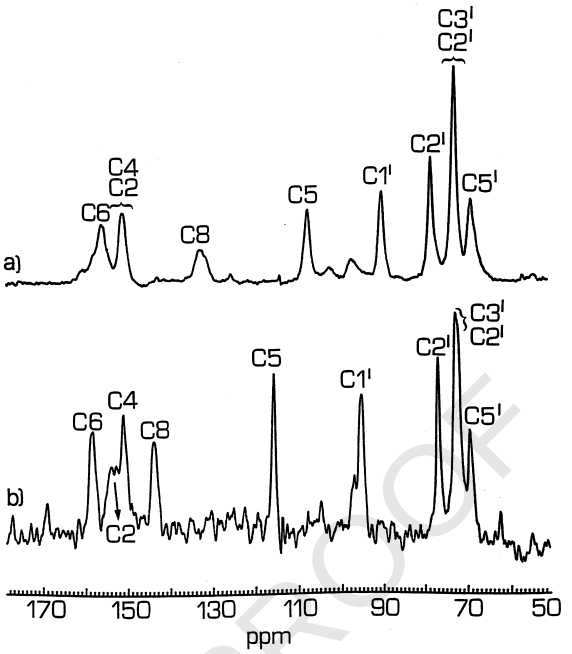

Fig. 5. CP-MAS ^{13}C NMR spectra of solid: (a) cGMPH and (b) cGMPNa.

spectra of cGMPH (a) and cGMPNa (b), respectively (to our knowledge not yet reported in the literature) and their relative assignments of guanine ring and sugar carbons. The most evident data are: the shift of the C8 resonances from 132.7 in the acid to 144.0 ppm in the salt, of the C5 from 107.9 to 116.0 ppm and of C1′ from 90.3 to 95.5 ppm (see Table 1).

4. Discussion

The ^{1}H NMR spectra of cGMPH and cGMPNa in DMSO-d_6 show the same resonances as the guanine ring protons. Interestingly, the ^{1}H spectra show a large shift of 1.3 ppm of the H1′ resonance: in our opinion this behaviour indicates a different conformation of the sugar ring in the two compounds.

Analogously, the ^{13}C NMR spectra of cGMPH and cGMPNa in DMSO-d_6 show the coinciding chemical shifts of the guanine resonances (differences less than 0.3 ppm are within the experimental error) and, in particular that at 116.5 ppm of the C5, indicates that the guanine rings in both

compounds are not N7-protonated [4]. Thus the difference of 2 ppm in the ^{31}P spectra (Fig. 2) indicates two phosphate groups with a different ionization degree. Therefore, in the case of the acid in DMSO-d_6, it can be concluded that the proton is localized on the phosphate group. Indirect evidence is also given by the differences of chemical shift of C3′ and C5′, adjacent to the phosphate group, of cGMPH and cGMPNa. This conclusion is strongly confirmed by the Raman spectra in DMSO-d_6 where the bands in the range 1250–1750 cm^{-1} (Fig. 3), in particular the 1490 cm^{-1} band, typical of the free N7 guanine ring [7] coincide both for the acid and salt solutions.

The data observed for the ^{13}C and ^{31}P NMR spectra of cGMPNa following the addition of 1 and 2 equivalents of HCl show that in the first step the protonation of the phosphate group occurs, in the second step the further proton interacts with the N7 of the guanine ring. In fact, the upfield and downfield shifts observed for the guanine carbons following addition of 2 HCl equivalents are similar to those observed by us in the case of the N7 protonation of Guo [4].

Interestingly, the spectroscopic data in water, if compared with those in DMSO, show a different behaviour of the two cyclic nucleotides. Indeed, the solvent seems to have a strong effect in determining the localization of the proton in cGMPH. In fact, contrary to the DMSO-d_6 solutions, ^{13}C and ^{31}P data in D_2O show that cGMPH has the proton localized on the guanine ring. In fact, the ^{13}C chemical shifts of cGMPH compared with those of cGMPNa show differences in agreement with the previously discussed N7 protonation. In addition, the ^{31}P data confirm that the phosphate is not protonated.

The Raman spectrum of cGMPH in aqueous solution shows an intensity behaviour of the 1490 and 1550 cm^{-1} bands in between those of cGMPH and cGMPNa in the solid state. A decrease in intensity of the 1490 cm^{-1} (ν_{10}) band and an increase in intensity of the 1550 cm^{-1} band (ν_{11}) suggest that the N7 site is protonated, in agreement with Perno et al. [7]for similar compounds. The results obtained from either Raman or NMR aqueous solution spectra indicate that, contrary to DMSO, the phosphate group is not protonated and the N7 position is partially protonated in agreement with the upfield shift of the C5 resonance from 113.8 (cGMPNa) to 108.6 ppm (cGMPH). These data suggest that in aqueous solution an equilibrium exists between the N7 protonated form and the dissociated one, depending on pH (see Fig. 4, cGMPH in aqueous acid solution where the 1550 cm^{-1} band is more intense than in aqueous solution), both characterized by a phosphate group (PO_2^-) with the same ionization degree. The ν_{11} Raman band from water to DMSO is shifted by about 20 cm^{-1}, probably due to a different interaction with the solvent.

Finally, from the examination of ^{13}C solid state spectrum of cGMPH an upfield shift of 8.1 ppm of C5 can be observed when the guanine ring is protonated, similar to that observed in the ^{13}C spectra of guanosine derivatives in DMSO-d_6 solution [4]. Interestingly, the C8 resonance moves upfield by 11.3 ppm owing to protonation. This behaviour can be considered a marker of N7 protonation and is not observed in the solution spectra probably due to cancelling factors [4]. Moreover, the C1′ resonance is particularly affected comparing the acid and salt spectra, a shift of more than 5 ppm indicating a different conformation of cGMPH sugar ring. From the spectrum it can be also desumed that the protonation occurs at the N7 position (as in aqueous solution) and the sugar conformation is different from that of the cGMPNa [8].

5. Conclusions

cGMPH behaves differently in water and DMSO solution. In DMSO the hydrogen is located on the phosphate group while in water it is mainly on the N7 guanine ring with an equilibrium depending on pH. The solvent therefore has a major effect on the protonation site. In DMSO, by addition of acid to cGMPNa in the first step, the phosphate group is protonated while the second proton is localized on the guanine ring, as in water. The spectroscopic characterization suggests that cGMPH in the solid state is N7 protonated, the phosphate group deprotonated and the sugar conformation differs from that of the salt. It is to notice that the N7 protonation of cGMPH both in water and in the solid state is in agreement with the pKa values for N7H and PO_2H groups ($pK_{N7H^+} = 2.4$; $pK_{PO_2H} = 1.5$) [9,10] while in DMSO solution the first

protonation occurs on the phosphate group, owing to the different interaction with the solvent.

Acknowledgements

This work was supported by grants of MURST (ex 60%). Thanks are due to the Italian CNR-I.Co.C.E.A. (Bologna) for the Solid State NMR facilities.

References

[1] H.H.H.W. Schmidt, S.M. Lohmann, U. Walter, Biochim. Biophys. Acta 1178 (1993) 153.
[2] V. Tugnoli, M.R. Tosi, G. Barbarella, A. Bertoluzza, R. Ricci, C. Trevisan, Anticancer Res. 16 (1996) 2891.
[3] C.S. Yannoni, Acad. Chem. Res. 15 (1982) 201.
[4] G. Barbarella, A. Bertoluzza, M.A. Morelli, M.R. Tosi, V. Tugnoli, Gazz. Chim. It. 118 (1988) 637.
[5] R.D. Lapper, H.H. Mantsch, C.P. Smith, J. Am. Chem. Soc. 95 (1973) 2878.
[6] Y. Nishimura, M. Tsuboi, T. Sato, K. Aoki, J. Mol. Struct. 146 (1986) 123.
[7] J.R. Perno, D. Cwikel, T.G. Spiro, Inorg. Chem. 26 (1987) 400.
[8] M.E. Druyan, M. Sparagna, S.W. Peterson, J. Cyclic Nucleotide Res. 2 (1976) 373.
[9] K.H. Scheller, V. Scheller-Krattiger, R.B. Martin, J. Am. Chem. Soc. 103 (1981) 6833.
[10] S.S. Massoud, H. Sigel, Inorg. Chem. 27 (1988) 1447.