THE SPECIFIC INTERACTION OF GUANINE WITH CARBOXYLATE IONS IN WATER

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1. Introduction

Several types of direct and indirect interactions have been shown to occur in protein–nucleic acid complexes: electrostatic interactions between lysine or arginine side chains and phosphate groups, stacking interactions of nucleic acid bases with aromatic amino acid residues and hydrogen bonding interactions. Among amino acid side chains, ionized carboxylate groups of glutamic and aspartic acids are the only ones which possess a functional group in the correct position to bind the N(1)H and N(2)H_2 groups of guanine [1]. On the other hand, guanine is the only base which has two acceptor groups in the correct position to form two hydrogen bonds with carboxylate anions. Studies of complex formation involving hydrogen bonding between nucleic acid bases and amino acid side chains have been already carried out in organic solvents such as cyclohexane [2], chloroform [3–5] and dimethylsulfoxide [6–8]. One of the most important results was to demonstrate that carboxylate ions interact strongly with guanine, only weakly with adenine, cytosine and uracil and that this interaction induced a dissociation of the guanine–cytosine base pair in dimethylsulfoxide [6]. Since the selective interaction between carboxylate groups of proteins and guanine bases might be an element of a molecular code for the recognition of nucleic acid base sequences by proteins the aim of this work was to investigate the interaction of carboxylate ions with nucleotides by proton magnetic resonance in water.

Abbreviations: Recommended by the IUPAC–IUB Commission on Biochemical Nomenclature. A summary of these recommendations may be found in Biochim. Biophys. Acta (1971) 247 1–12

2. Experimental

Nucleosides and nucleotides were purchased from Sigma. Sodium acetate was obtained from Merck. PMR spectra were recorded with a Bruker WH 90 spectrometer operating in the pulse Fourier transform mode. PMR spectra in water were obtained at 90 MHz with a low gain and a pulse of 1 μs. The 250 MHz spectra in water have been recorded on a Cameca TSN 250 equipped with a Nicolet 1180 computer. The use of long excitation pulses (333 μs) of weak intensity associated with a carrier frequency at 3000 Hz has led to an attenuation by a factor ~1000 of the H_2O resonance [9]. Temperature was measured with an accuracy of ±1°C and regulated to ±0.5°C. The pH of the solutions was measured in water and was assumed to be constant after addition of Me_2SO-d_6 or 20% D_2O. All chemical shifts were measured with respect to an internal reference either tetramethylsilane (TMS) in mixture Me_2SO/H_2O or sodium 3-(trimethylsilyl) propane sulfonate (DSS) in aqueous solutions.

3. Results

3.1. *Selective binding of carboxylate ions to guanine in water*

The solubility of guanine or guanosine is rather small in water. PMR measurements were therefore carried out with nucleosides 5′-monophosphate. The first indication of a specific interaction between carboxylate anions and Guo-5′-P comes from the observation that an aqueous solution of Guo-5′-P (0.2 M) precipitated by addition of 0.2 M sodium acetate at 275 K whereas mixtures of Ado-5′-P, Ura-5′-P or Cyd-5′-P formed clear solutions in the same conditions. To avoid precipitation in presence of carboxylate ions

the maximum concentration used for Guo-5′-P was 0.1 M. At 300 K, the PMR spectrum of Guo-5′-P shows 2 resonance lines at 8.164 ppm and 6.253 ppm assigned to H(8) and $N(2)H_2$ protons, respectively. Mixing Guo-5′-P (0.1 M) with sodium acetate (1 M) led to a downfield shift (0.15 ppm) of the amino proton resonance of guanine. The same behaviour has been already observed upon hydrogen bond formation between carboxylate anions and 9-ethylguanine in dimethylsulfoxide [6]. But in water the interpretation of such results is complicated by several possible association processes such as gel formation [10], stacking, interaction between phosphate and bases [11]. At pH 7.1 an increase of the concentration of Guo-5′-P from 0.028–0.11 M led to an upfield shift of the NH_2 resonance by 0.007 ppm at 275 K. Therefore, the downfield shift observed for NH_2 protons in the presence of acetate ions does not reflect the effect of destaking or competition with gel formation or phosphate–base interaction. The role of ionic strength was also studied. Adding 1 M sodium perchlorate left invariant the chemical shift of amino protons of Guo-5′-P. These results support the assumption that carboxylate ions bind to amino protons of Guo-5′-P in water.

At 300 K, addition of 1 M sodium acetate to an aqueous solution of Ado-5′-P or Cyd-5′-P left invariant the chemical shift of their amino protons. In water [12] or in Me_2SO containing small amounts of water [6], the N(1)H resonance line of derivates of uracil is too broad to be observed. Therefore, the possible interaction of carboxylate ions with Ura-5′-P in water was checked by competition with Guo-5′-P. At 300 K, the amino proton resonance line of 0.1 M Guo-5′-P is downfield shifted by 0.15 ppm in the presence of 1 M sodium acetate and by 0.16 ppm upon further addition of 0.1 M Ura-5′-P to this mixture. This result indicates clearly that the interaction of Ura-5′-P with carboxylate ions is small as compared to that of Guo-5′-P.

In conclusion, Guo-5′-P is the only nucleotide to interact strongly with carboxylate ions. As a matter of fact, guanine is the only base to possess 2 hydrogen bond donor groups [N(1)H and $N(2)H_2$] in the correct position to form a complex with carboxylate groups involving 2 hydrogen bonds (fig.1).

3.2. *Competition between sodium acetate and Cyd-5′-P for binding to Guo-5′-P in water*

In water, the complexation of Guo-5′-P by Cyd-5′-P has already been demonstrated [12]. In this complex

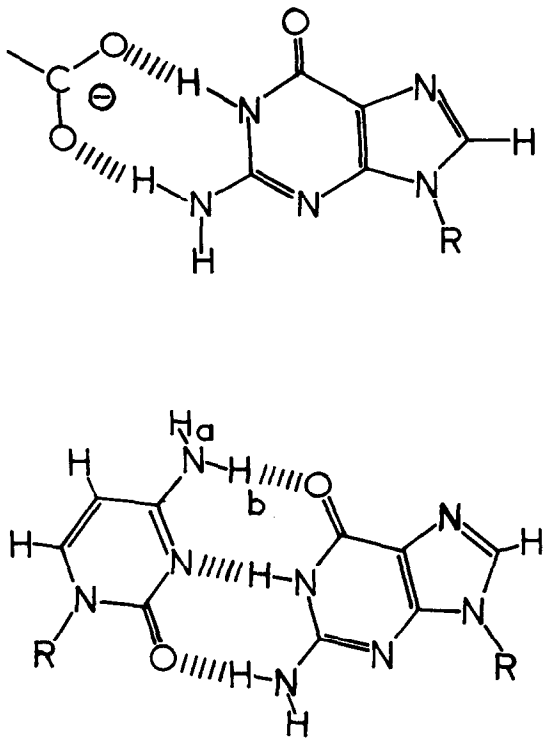

Fig.1. Scheme of the carboxylate–guanosine and cytosine–guanine hydrogen-bonded complexes.

as well as in the Guo-5′-P–carboxylate complex described above (fig.1) the N(1)H and $N(2)H_2$ protons of guanine participate in hydrogen bond formation. The binding site of carboxylate ions on guanine involves two groups which are free only if guanine is in a single-stranded nucleic acid. In double-stranded nucleic acids, the carboxylate binding site of guanine is not available except if protein binding produces a local disruption of base pairing. So, it was of interest to determine which of the G–C or G–carboxylate complexes was the strongest. Since in water, the association constants are too low to be computed with precision, the comparison has been made by competition experiments. Fig.2 shows the PMR spectra of Guo-5′-P (0.2 M), Cyd-5′-P (0.2 M) and their equimolar mixture in the presence or in the absence of sodium acetate (0.2 M) at 275 K in water. Mixing of Cyd-5′-P and Guo-5′-P led to a downfield shift of the NH_2 resonance line of Guo-5′-P and of the NH_b amino resonance of Cyd-5′-P (table 1, fig.2). Adding 0.2 M sodium acetate to this mixture downfield shifted the NH_2 resonance of Guo-5′-P. Although

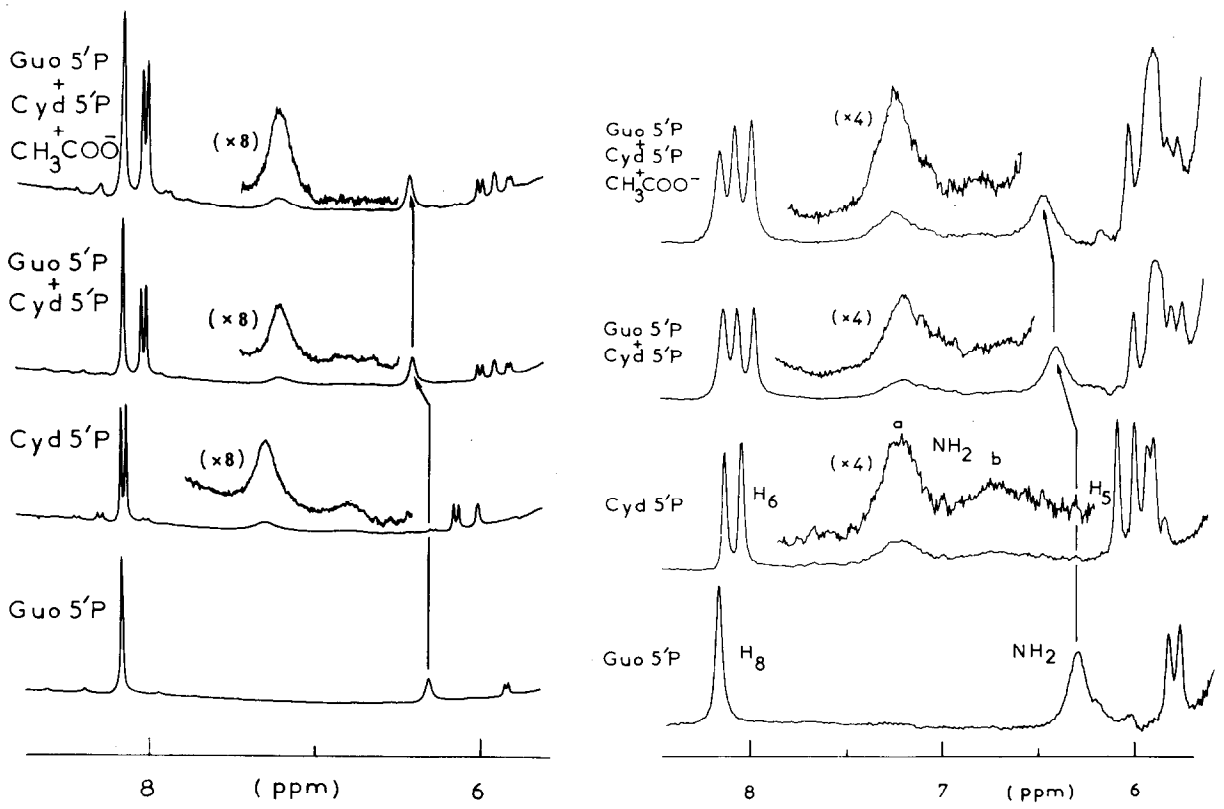

Fig.2. PMR spectra of at 90 MHz (right) and 250 MHz (left) Guo-5′-P, Cyd-5′-P and of their mixtures with or without sodium acetate in H_2O:D_2O (4:1) chemical shifts are measured with respect to DSS at 275 K. All concentrations are 0.2 M.

Table 1
Change in chemical shifts of the amino proton resonances of Guo-5′-P, Cyd-5′-P and of mixtures of Guo-5′-P, Cyd-5′-P and sodium acetate in H_2O:D_2O (4:1) solutions at pH 7.6 and 275 K

	$\delta NH_2(G)$	$\delta NH_2(C)$	
		H_b	H_a
Guo-5′-P + Cyd-5′-P	0.124	0.071	0.019
Guo-5′-P + Cyd-5′-P + CH_3COONa	0.150	0.065	0.000
Guo-5′-P + CH_3COO Na[a]	0.032		
Cyd-5′-P + CH_3COO Na		−0.019	0.000

[a] Change in chemical shift observed at 285 K for 0.1 M of Guo-5′-P and 0.1 M sodium acetate concentrations

Resonance lines of the amino group of Guo-5′-P and Cyd-5′-P are found at 6.351, 7.257 (H_b) and 6.775 (H_a), respectively. All concentrations (nucleotides and sodium acetate) are 0.2 M

these results are in agreement with the conclusions reached in Me_2SO solutions [6] (carboxylate ions bind NH_2 of Guo-5′-P in presence of Cyd-5′-P), the variation of the chemical shift of the NH_b of Cyd-5′-P in presence of Guo-5′-P and carboxylate ions is too small to conclude to a dissociation of the G–C base pair in water in presence of carboxylate ions. In fact the NH_b resonance line is broad and in water its downfield shift is small in presence of Guo-5′-P as observed in [12]. So the upfield shift expected in the presence of carboxylate ions cannot be important as shown by the 250 MHz spectra. Experiments in mixed solvents (H_2O/Me_2SO) were therefore carried out to determine guanine–carboxylate and guanine–cytosine association constants as a function of the water content.

3.3. *Hydrogen bond formation between guanosine and sodium acetate or cytosine in water–dimethylsulfoxide mixtures of different composition*

Guo-5′-P and Cyd-5′-P bear two negative charges on

Table 2
Chemical shifts of proton resonances of guanosine, cytidine and mixtures of guanosine, cytidine and sodium acetate in Me_2SO-d_6:H_2O (3:1) at 295 K and pH 7.0

	NH(G)	NH_2(G)	NH_2(C)	
			H_b	H_a
Guo	10.980	6.546		
Cyd			7.055	7.420
Guo + Cyd	11.307	6.709	7.283	7.492
Guo + Cyd + CH_3COO Na		6.886	7.075	7.492
Guo + CH_3COO Na		6.664		
Cyd + CH_3COO Na			6.996	7.394

Concentrations are 0.1 M in nucleosides and 0.2 M in sodium acetate

their phosphate groups at pH 7.0 and have rather low solubility in Me_2SO. Experiments have been carried out with the nucleosides (guanosine and cytidine) which are soluble up to 0.2 M in Me_2SO-d_6. Determination of the association constants has been made by fitting Job plots [13–15]. The large downfield shift of the NH_2 resonance of guanosine in the presence of sodium acetate (table 2) was used to determine the

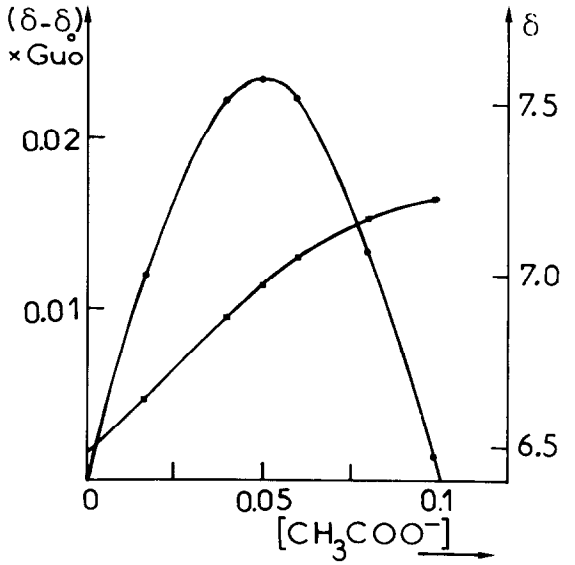

Fig.3. Job plot (•) for PMR data (NH_2 resonance) obtained with mixtures of Guo and sodium acetate in Me_2SO:H_2O mixture ([H_2O] = 2.7 M) at 295 K. The sum of solute concentrations was constant (0.1 M). The maximum of complex (proportional to $(\delta - \delta_0) \times$ [Guo]) was observed when sodium acetate concentration was equal to guanosine concentration (5×10^{-2} M each) demonstrating the formation of a 1:1 complex. The best fit obtained by using a least squares program led to $K = 51 \pm 5\ M^{-1}$ and $\Delta\delta_c = 0.87 \pm 0.05$ ppm. The variations of the chemical shift of Guo are given by (▪).

binding parameters. For all water–Me_2SO mixtures complex formation was maximum for equal concentrations of the two reactents ([acetate] = [guanosine]). The plot of fig.3 shows clearly the formation of a 1:1 complex as already reported in the presence of small amounts of water [6]. The association constant for complex formation was determined from a least-square analysis of changes in chemical shifts [4]. Table 3 gives the binding constants computed for the systems guanosine–cytidine–sodium acetate. It appears clearly that for all water contents, guanosine binds more strongly to carboxylate ions than to cytidine. Fig.4 shows the competition between cytidine and sodium acetate for binding to guanosine in 13.7 M water. These data support the conclusion that carboxylate ions are able to dissociate the G–C base pair:

4. Conclusion

We have shown that carboxylate anions interact only with guanine derivatives in water. The binding site of carboxylate ions to guanine involves two donor groups which are free only if guanine is in a single stranded nucleic acid. Since carboxylate ions bind more strongly to guanine than cytosine does, they might locally disrupt base pairing. Of course, disrup-

Table 3
Association constants for the binding of guanosine to cytidine (K_{G-C}) and of guanosine to sodium acetate (K_{G-COO^-}) at 295 K dimethylsulfoxide solutions containing different amounts of water

[H_2O]	0.6 M	2.7 M	5.7 M	13.7 M
K_{G-C} (M^{-1})	3.3	2.8	2.3	1.6
K_{G-COO^-} (M^{-1})	85	51	27	18

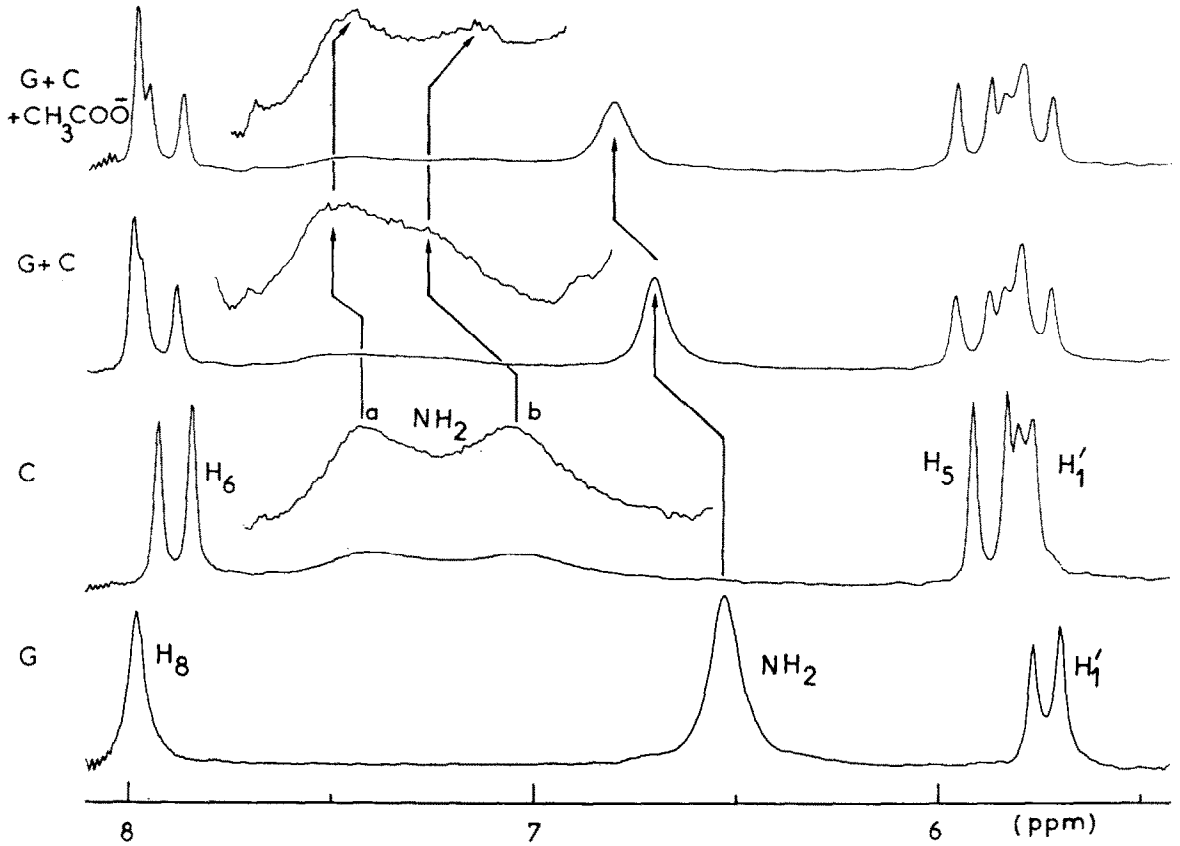

Fig.4. PMR spectra of Guo, Cyd and of their mixture in the presence or the absence of sodium acetate in $Me_2SO:H_2O$ (3:1) at 295 K and pH 7.0. Chemical shifts are measured with respect to an internal TMS reference. Concentrations are 0.1 M in nucleosides and 0.2 M in sodium acetate.

tion of a guanine–cytosine base pair in a double-stranded nucleic acid clearly requires stronger energy than for an isolated base pair due to the stacking energy with neighbouring base pairs and to the rotational restrictions of the phosphodiester bonds. The possibility of hydrogen bonding association between glutamic acid (or aspartic) side chains with guanine and G–C base pairs is presently investigated using oligopeptide–oligonucleotide complexes. The selective interaction of carboxylic acid side chains of proteins with guanine bases might represent one element of a molecular code for the recognition of nucleic acid base sequences by proteins.

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