NMR EVIDENCE OF SPECIFIC BASE-PAIRING BETWEEN PURINES AND PYRIMIDINES

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NMR studies of the purine and pyrimidine components of nucleic acids have been reported by a number of investigators (1-10). The NMR spectra of random coil polynucleotides have also been observed (11), but proton signals of helical complexes (eg. DNA, poly (A+U))*(11,12) are not observed because of the line broadening which occurs in large molecules with slow rates of tumbling. The occurrence of interaction between smaller molecules, however, should circumvent this latter difficulty and permit observation of proton NMR spectra in base-paired complexes (13).

We report here NMR evidence that mixtures of simply substituted purines and pyrimidines in DMSO solution interact to form base-paired complexes and that the specificity of pairing appears to be similar to that observed for polymer-polymer (14) or monomer-polymer (15) interactions in aqueous solution.

It is well known that hydrogen bonding results in the shift of the proton signal to lower field (16). Although the organic solvents employed (DMSO and a mixture of this solvent with DMF) are known to be

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*The following abbreviations are used: DNA, deoxyribonucleic acid; poly (A+U), complex found between polyadenylic and polyuridylic acids; DMSO, dimethylsulfoxide-d$_6$; DMF, dimethylformamide-d$_7$; AA, 2-aminoadenosine; A, 9-ethyladenine; C, 1-methylcytosine; G, 9-ethylguanine; T, 1-methylthymine.

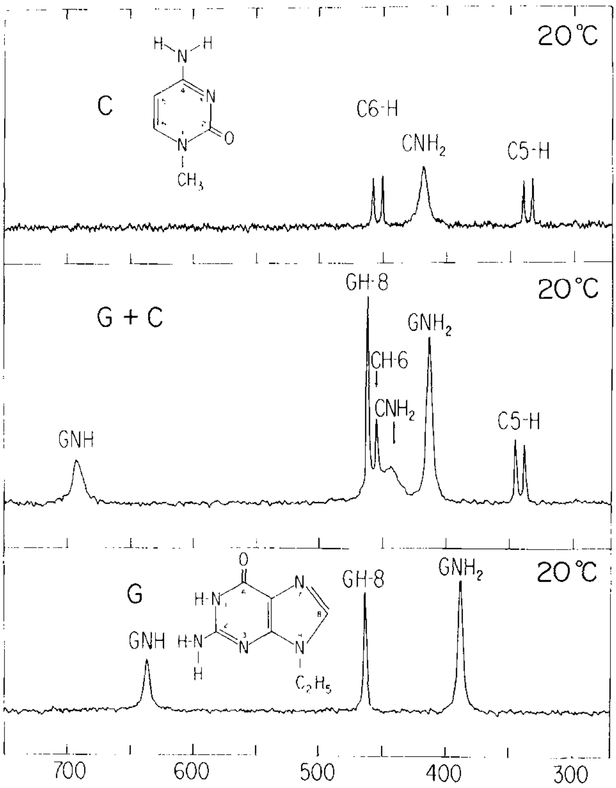

Fig. 1. NMR spectra of 9-ethylguanine (G) and 1-methylcytosine (C) in DMSO-d$_6$. The spectra were measured with a Varian A-60 spectrometer. Each spectrum was calibrated by means of audio frequency side bands. The abscissa is given in cps as downfield shifts from an internal TMS standard.

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hydrogen bond acceptors (17), we can take as our reference state the one in which purine or pyrimidine is bonded to solvent and look for further downfield shifts of the NH and NH$_2$ protons upon admixture with a potentially pairing purine or pyrimidine. The basic observation is that this admixture does result in further downfield shifts for certain pairs and that the magnitude of the shift increases with concentration and decreases with temperature as compared with the two bases measured separately at the same concentrations and temperatures. Those pairs for which this hydrogen bonding shift occurs are those known to interact as polymers in aqueous solution, and the NH and NH$_2$ signals of the other pairs so far observed undergo no shift upon mixing (Table I). Similarly there is no appreciable change with concentration of the chemical shift of the NH and NH_2 protons of the individual bases under the conditions studied (Table II). The aromatic CH protons do not exhibit upon mixing the pattern of upfield shifts which were shown by Chan *et al.*(9) to result from vertical stacking of the bases. It seems probable, therefore, that such stacking does not occur to a major extent under the conditions of our experiments.

Fig. 1 illustrates the shifts the NH and NH_2 protons undergo when 1-methylcytosine and 9-ethylguanine are mixed in equimolar amounts in DMSO solution. From this figure and from Table I it will be seen that these shifts vary with temperature and concentration in the manner expected for the formation of a hydrogen bonded complex.

Table I shows that the magnitude of the change of the G-NH frequency upon mixing is twice that of both C and G amino groups, and that this 2:1 ratio of the magnitude of the shifts is maintained at all temperatures and concentrations observed. This ratio is consistent with a time averaging of the shifts of the two protons in the amino group (one bonded to the other base and one bonded to the solvent), and suggests that the base pairing shift is approximately the same for all the hydrogen bonding protons in the GC complex.

The use of a 1:1 mixture of DMSO and DMF as the solvent made it possible to study the C-G complex formation at temperatures down to -10°C. Methylcytosine labelled with ^{15}N in the amino group (7) provided a means for clear observation of the C-NH_2 signal, since the low field part of the C-$^{15}NH_2$ doublet [$J(^{15}N\text{-}H) \sim 90$ cps] now was unobscured by the signals from the aromatic protons. The low field part of the C-NH_2 signal is very broad at room temperature and is split into 2 peaks at lower temperatures (see Fig. 2 and Table I), evidently due to hindered rotation of the amino group. Upon changing the GC molar ratio from 1:1 to 1:2 we find that the interaction shifts of each of the C amino protons is precisely half that in the

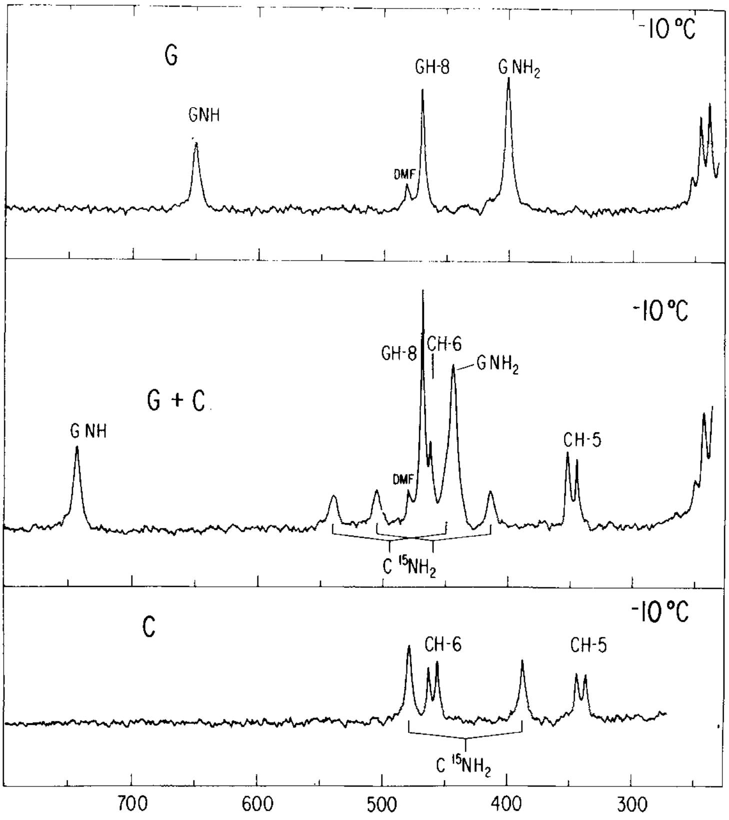

Fig. 2. NMR spectra of 9-ethylguanine (G) and 1-methylcytosine-7-^{15}N(C) in DMSO-d_6:DMF-d_7 (1: 1).

1:1 mixture. This observation demonstrates that the rate of interchange between complexes and uncomplexed bases is sufficiently rapid to permit averaging of the chemical shifts. The apparent absence of splitting in the case of C alone may result from accidental coincidence of the chemical shifts of the two amino protons. The factors involved in this effect, as well as the difference in the behavior between G and C amino groups, are under investigation.

A mixture of 1-methylthymine and 9-ethyladenine shows a downfield shift of T-NH but virtually no shift of A-NH$_2$. Similar but somewhat larger shifts are observed for mixtures of 1-methylthymine and 2-aminoadenosine,

TABLE I. Interaction shifts on base pairing[a,b]

Bases	Conc (M)	T (°C)	Interaction Shift (ppm) G-NH	G-NH$_2$	C-NH$_2$	T-NH	A-NH$_2$
G+C	.1 + .1	40	0.5	0.24	0.29		
		20	.71	.34	.36		
	.2 + .2	40	.7	.34	.46		
		20	.94	.41	.43		
A+T	.1 + .1	20				.05	.02
	.2 + .2	20				.08	.02
AA+T	.2 + .2	20				.15	.05[d] .07
G+T	.2 + .2	20	.00	.00		.00	
G+A	.2 + .2	20	.02	.00			.00
C+T	.2 + .2	20			.03	.00	
G+C	.2 + .2	40[c]	1.1	.52	.5		
		20[c]	1.29	.59	.5		
		2[c]	1.46	.66	.43 .96		
		-10[c]	1.57	.72	.44 1.02		
	.1 + .2	-10[c]	1.73	.82	.24 .51		

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[a] All measurements made in DMSO-d_6 solution except where indicated.

[b] No interaction shift was observed for G-8H; a small downfield shift (≤ 0.1 ppm) was found for C-5H and C-6H.

[c] In DMSO-d_6-DMF-d_7 (1:1 by volume)

[d] The .05 and .07 ppm refer to the interaction shift of 6-amino and 2-amino group, respectively, of 2-aminoadenosine.

which are stereochemically capable of forming three interbase hydrogen bonds. The relationship of interaction shifts (GC >> AAT > AT) is consistent with the order of stability observed for helical complexes in

TABLE II. Chemical Shifts of Base Protons[a]

Solvent	Conc (M)	T(°C)[c]	δ (ppm from TMS)[b]				
			G-NH	G-NH_2	C-NH_2	T-NH	A-NH_2
DMSO	0.1	40	10.47	6.37	6.87		
		20	10.54	6.44	6.96	11.24	7.22
	.2	40	10.51	6.40	6.86		
		20	10.60	6.48	6.97	11.24	7.22
DMSO-DMF	.2	40	10.5	6.42	6.9		
		20	10.67	6.53	7.03		
		2	10.75	6.63	7.14		
		-10	10.82	6.68	7.23		

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[a] Chemical shifts of aromatic C-H protons are constant within experimental error, with change of temperature or concentration in DMSO. In DMSO-DMF a small temperature effect (< 0.1 ppm over a 50° range) was observed.

[b] Experimental uncertainty is estimated to be 1 cps or ca. 0.02 ppm except for broad peaks.

[c] The temperature measurements are estimated to be accurate within 1°.

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aqueous solution, and suggests that other factors in addition to the number of hydrogen bonds are of major importance in determining complex stability.

When these same bases are mixed in pairs which do not interact in the nucleic acids or polynucleotides, the chemical shifts of the bases in the mixture are the same as those of the two components measured separately under the same conditions. Thus, the GT, CT, and AG pairs show no changes in spectrum upon mixing and hence no evidence of interaction.

These NMR results strongly support a Watson-Crick GC pairing since they show that both amino groups and the G-NH are all involved in specific hydrogen bonding, a conclusion which is inconsistent with other bonding schemes (18). Similarly we conclude that proton transfer may be excluded since the spectra of the mixtures are not those to be expected from a mixture of cytosine cations and guanine anions. The possibility of change of tautomeric form upon pair formation may be ruled out by the spectra in Fig. 2 which show $^{15}NH_2$ spin-spin coupling in the complex as well as in C alone and hence that C is in the amino form.

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