Hydrogen Bonding Specificity of Nucleic Acid Purines and Pyrimidines in Solution

Abstract. *The hydrogen bonding of derivatives of guanosine and of cytidine soluble in chloroform has been studied in the infrared. These molecules associate with themselves, but from stronger complexes with each other. Earlier work showed that a similar association occurs between derivatives of adenine and of uracil. A study of all possible interactions of these purines and pyrimidines as pairs shows that guanosine and cytidine and adenine and uracil are the only associating, mixed dimer pairs. These interactions thus reinforce the geometrical specificity seen in the two-stranded nucleic acids.*

Information in biological systems is stored in the linear sequence of nucleotides in nucleic acid molecules. An essential process in the replication and distribution of this information is the formation of complementary structures in which the nucleotide bases are paired with each other through a system of specific hydrogen bonding. This is seen most clearly in the double-stranded form of DNA, in which adenine forms hydrogen bonds with thymine and guanine bonds with cytosine. Similar types of specific interaction are believed to take place when DNA directs the formation of RNA, except uracil then replaces the thymine residue. Specific hydrogen bonding is involved in the interaction of nucleic acids during protein synthesis. Thus, these interactions are fundamental in biological systems.

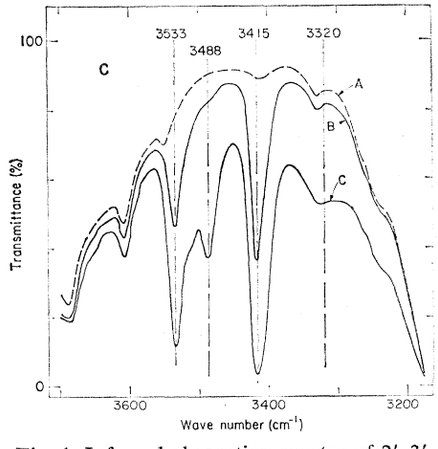

Fig. 1. Infrared absorption spectra of 2′, 3′-benzylidine-5′-trityl-cytidine in deuterochloroform recorded on a Perkin-Elmer Model 521 double-beam spectrometer with path length of 1 cm. The dashed curve *A* is the absorption spectrum of the solvent itself, curve *B* the spectrum of 0.0016*M* solution, and curve *C* that of 0.008*M* solution. The strong bands at 3533 and 3415 cm^{-1} represent the antisymmetric and symmetric NH stretching vibrations associated with the free amino group of cytidine. The association bands at 3488 and 3320 cm^{-1} (curve *C*) are due to hydrogen bonding of the amino group. The cytidine and guanosine derivatives were purchased from Cyclo Chemical Corporation, deuterochloroform from New England Nuclear Corporation.

Because it is possible to co-crystallize derivatives of the complementary bases, the selectivity of hydrogen bonding between purines and pyrimidines can be shown rather clearly in the solid state. X-ray analysis of these structures shows hydrogen-bonded pairs in which residues of guanine and of cytosine, as well as of adenine and thymine (or uracil), are associated (*1*). The analogous association of derivatives of uracil and of adenine in chloroform solution as revealed by their infrared spectra has already been described (*2*). A nonpolar solvent was chosen for this work because it allowed observation in the infrared of the stretching vibrations of the NH groups directly involved in hydrogen bonding. It also enabled a more reliable determination of the association energy because hydrogen bonding by the solvent is of minor importance. Moreover such a solvent eliminates the "vertical stacking" of purines and pyrimidines, which is believed to occur in aqueous solutions (*3*) as a result of hydrophobic interactions between the aromatic ring systems.

The infrared studies showed that the derivatives of adenine and of uracil are both capable of associating with themselves through hydrogen bonding. However, when they are mixed together, they associate with each other much more strongly.

The association constant of adenine and uracil is some 20 times greater than that of adenine and adenine or of uracil and uracil (*4*). Thus, these derivatives show the same type of specificity as the nucleic acids themselves. Furthermore, the fact that the association between adenine and uracil involves two hydrogen bonds, as do the associations of adenine with adenine and of uracil with uracil, suggests that there is another component of the interaction energy to be considered. A similar finding was reported independently by Kuechler and Derkosch (*5*), who carried

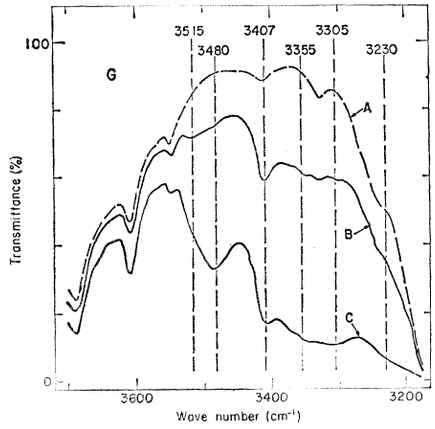

Fig. 2. Infrared spectra of 2′,3′-benzylidine-5′-trityl-guanosine in deuterochloroform (path length 1 cm). Curve *A* is the absorption spectrum of the pure solvent while curves *B* and *C* are the spectra of 0.0016*M* and 0.008*M* solutions of the guanosine derivative. Bands due to nonbonded antisymmetric and symmetric NH stretching vibrations appear at 3515 and 3407 cm^{-1}, while the bands at 3480, 3355, 3305, and 3230 cm^{-1} are association bands due to the hydrogen bonding of the guanine residues with themselves. Even in the more dilute solution (curve *B*) the association bands are observable.

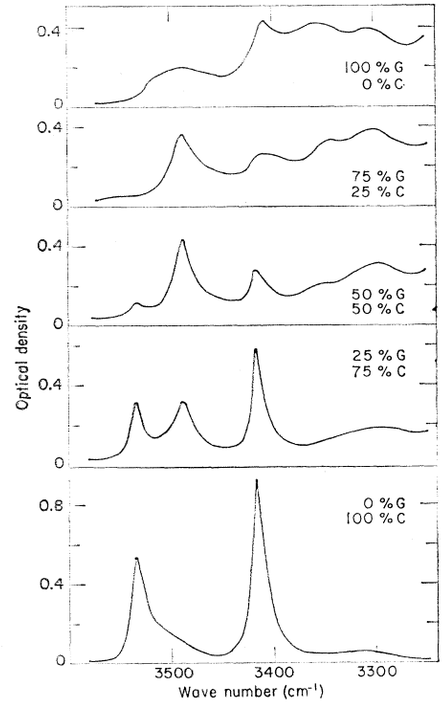

Fig. 3. Infrared absorption spectra of various mixtures of 2′, 3′-benzyldine-5′-trityl-derivatives of guanosine and cytidine (path length 2.5 cm). The total concentration of material is 0.0016*M*, but the mole ratio varies as indicated in the figure. By plotting optical density rather than the percentage of transmittance (as in Figs. 1 and 2), and by using solvent absorption as the base line, one is able to see the solute absorption more clearly. Strong association bands arise in the 1:1 mixture of guanosine and cytidine derivatives.

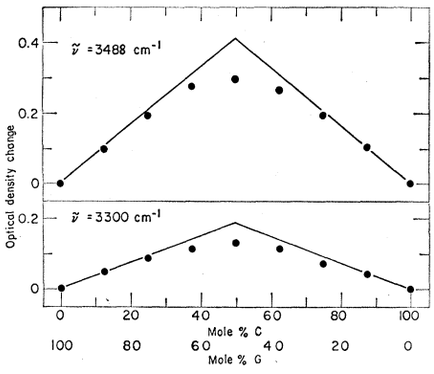

Fig. 4. Change in the optical density of the association bands found at 3488 and 3300 cm^{-1} as a function of mole ratio of guanine and cytosine derivatives. The optical density of the pure monomers is adjusted to 0, and the total concentration of solutes is 0.0016*M* for all points. It can be seen that the change in optical density is a maximum at 1 : 1 mixture of the guanine and cytosine derivatives in both bands. The solid line is drawn to indicate the change in optical density which would occur if the association constant for the guanosine and cytosine derivatives were very high. Deviations from this allow us to estimate a probable association constant of the order of 10^4.

out infrared studies of other derivatives of adenine and uracil in a different solvent. Nuclear magnetic resonance (NMR) studies of the same phenomenon have been performed by Katz and Penman (*6*) and by Shoup, Miles, and Becker (*7*), who demonstrated these associations by shifts in the proton signals for a variety of purine and pyrimidine derivatives in several solvents.

We have studied the association of guanine and cytosine derivatives in chloroform. The results augment our earlier findings which suggest a remarkable specificity in the association of monomeric purine and pyrimidine residues in solution. Guanosine and cytidine, although normally insoluble in a nonpolar solvent, will dissolve in chloroform if hydrophobic groups are added onto the sugar residue. We used 2′, 3′-benzylidine-5′-trityl-guanosine and the correspondingly substituted cytidine which have a solubility in chloroform of 0.5 and 0.01 mole/liter at 23°C. This solubility is sufficient to give adequate absorption in the region of the stretching vibrations of the NH group.

The infrared spectrum of the cytidine derivatives is shown in Fig. 1. Association bands occurring at 3488 and 3320 cm^{-1} represent the change in the stretching frequencies of the NH groups associated with the formation of hydrogen bonds (curve *C*). In this case, it is clear that the cytosine derivative is self-associating at the higher concentration (.008*M*), but only very little self-association is observed at the lower concentration (.0016*M*).

The infrared absorption spectrum of the guanosine derivative (Fig. 2) is somewhat more complicated, since the molecule shows evidence of considerable self-association even at the lower concentration (0.0016*M*). The guanine residue has an amino group which gives rise to antisymmetric and symmetric stretching vibrations at 3515 and 3407 cm^{-1}. In addition, it has an NH stretching vibration associated with the ring nitrogen, which is probably found in the band at 3407 cm^{-1}. However, as shown by curve *B*, the antisymmetric stretching vibration at 3515 cm^{-1} is not very intense. At the higher concentration this band can hardly be seen, but strong association bands can clearly be seen at 3480, 3355, 3305, and 3230 cm^{-1}. It is not surprising that the guanosine derivative associates strongly in dilute chloroform solution since guanylic acid residues can form a stable macromolecular complex even in aqueous media (*8*).

Although the guanosine derivative associates strongly with itself in dilute solution, it can form hydrogen bonds with the cytosine residue even more vigorously (Fig. 3). The spectra of various mixtures of guanine and cytosine derivatives are plotted in absorbance units from which the solvent absorbance has been subtracted in order that the absorption bands may be more clearly seen. The spectrum of the pure guanine derivative shows evidence of self association, as illustrated in Fig. 2. The absorption spectrum of the cytosine derivative is shown with the two prominent bands associated with the stretching vibrations of the amino group. Strong association bands are clearly visible in the 1 : 1 mixture of guanine and cytosine residues. The intensity of the association bands at 3488 and 3300 cm^{-1} changes as a function of the mole ratio (Fig. 4). The intensity of the bands increases to a maximum in the 1 : 1 region. These results are clearly similar to those shown previously for the adenine and uracil derivatives, which also preferentially associate by way of hydrogen bonds (*9*).

The availability of adenine, uracil, guanine, and cytosine derivatives which are all soluble in the same solvent allows us to study all of the interactions of these bases, as has been done in the NMR studies (*6, 7*). From Fig. 5, it is clear that guanine and cytosine interact strongly with each other, whereas the observed absorption spectrum of guanine and adenine derivatives is vir-

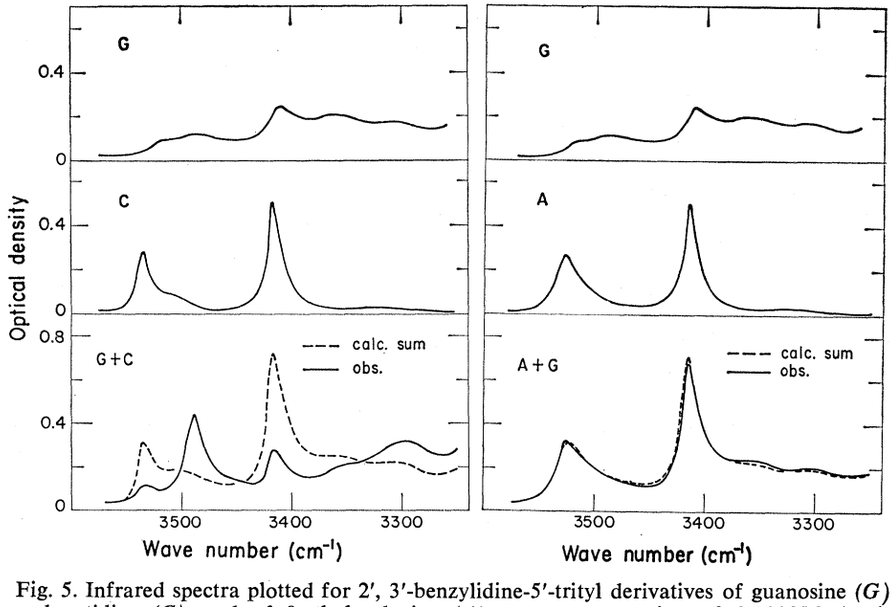

Fig. 5. Infrared spectra plotted for 2′, 3′-benzylidine-5′-trityl derivatives of guanosine (*G*) and cytidine (*C*), and of 9-ethyl adenine (*A*) at a concentration of 0.0008*M* (path length 2.5 cm). The dashed line is the calculated spectrum representing the sum of the upper two spectra obtained if the molecules did not interact. The solid line is the observed sum when these two solutes are mixed together so that they are each present in the solution at a concentration of 0.0008*M*. In the case of the guanosine and cytosine derivatives, it can be seen that the observed spectral differ considerably from the calculated sum and that strong association bands occur in the solution. In the case of the adenine and guanine derivatives, the observed spectrum is very close to the calculated sum which would be anticipated for noninteracting solutes.

Table 1. Ratio of optical densities of dimer bands to those of monomer bands. Observed and calculated ratios are listed for various mixtures of 2′,3′-benzylidine-5′-trityl derivatives of guanosine (G) and cytidine (C), 9-ethyl adenine (A) and 1-cyclohexyl uracil (U) in chloroform at 25°C. The upper figure in each square is the observed ratio of the optical density of the dimer band to that of the monomer band. The dimer band used is that which appears between the symmetric and antisymmetric stretching bands of the monomer. The lower figure (in parenthesis) is the calculated ratio which is obtained from the sum of the optical densities of the individual (unmixed) solutions at half the total concentration. The observed and calculated ratios are similar in all cases with the exception of those determined for the interaction between the guanosine and cytidine derivatives and between the adenine and uracil derivatives. The results are presented for two different total concentrations.

Derivative	Ratio of optical densities for derivatives		
	C	U	G
*Total concentration, 0.0016*M			
A	0.20 (.21)	0.32 (.23)	0.48 (.50)
G	3.7 (.56)	1.2 (1.1)	
U	0.27 (.28)		
*Total concentration, 0.0080*M			
A	0.30 (.29)	0.55 (.23)	0.53 (.55)
G	5.4 (.70)	1.3 (1.4)	
U	0.34 (.32)		

tually identical to the sum of the two individual spectra. These two molecules are present in solution, but they do not associate with each other to any observable extent. This is remarkable since both adenine and guanine have donor protons and electronegative atoms which are capable of forming hydrogen bonds. However, there is no preferential association.

Table I, listing the ratio of the optical density of an association band to that of a monomer band, gives the results of all possible mixtures. For example, in the spectrum of the mixed guanosine and cytidine derivatives (Fig. 5), this ratio is that of the optical density of the mixed dimer band at 3488 cm^{-1} to the optical density of the monomer band at 3533 cm^{-1}. The ratio of the analogous optical densities obtained by adding the optical densities of the separate solutions at half concentration is also given. The two ratios would be equal in all cases if there were no interaction other than self-association. These calculated and observed ratios are substantially the same for mixtures of derivatives of adenine with cytosine, adenine with guanine, adenine with cytidine, guanine with uracil, and cytosine with uracil. However, a substantial difference between observed and calculated ratios is seen in the mixtures of the derivatives of adenine and uracil and of those of guanine with cytosine.

These results together with those of NMR studies clearly illustrate the fact that adenine and uracil (or thymine) derivatives associate with each other selectively, as do derivatives of guanine and cytosine, a feature of the selectivity found in the nucleic acids themselves. One cannot directly relate the studies in nonaqueous solution to the interactions found in aqueous solutions, but since the interior of the double-stranded DNA molecule is largely hydrophobic, it is not unreasonable to assume that this type of interaction operates within the DNA molecule.

It is widely believed that complementary pairing of purines and pyrimidines in the nucleic acids is largely due to the geometry of the polynucleotide backbone, since these pairs alone give rise to a unit which is able to fit into a regular double-stranded helix. However, now we would say that the double-helical form of the nucleic acid molecule represents a combination of both geometrical requirements and other stabilizing features which we have observed. It has been suggested (*2*) that forces between dipoles or between dipoles and induced dipoles arising within the unsaturated purine and pyrimidine rings may contribute significantly to the specificity of these interactions. Further investigation will be necessary before the nature of these forces is completely elucidated.

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9. Recently we have learned of an infrared study [J. Pitha, R. N. Jones, P. Pithova, *Can J. Chem.* **44**, 1045 (1966)] of other derivatives of guanosine and cytosine in chloroform solution. Their results are consistent with the observations reported here and with those of NMR studies.
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Anticholinergic Blockade of Centrally Induced Thirst

Abstract. *A cholinergic antagonist applied to many of the limbic and diencephalic structures, cholinergic stimulation of which induces thirst, prevents drinking in response to cholinergic stimulation of other parts of the system. Thus, the elicitation of drinking after chemical stimulation of a particular site seems to be dependent on the functional state of other structures in the circuit.*

The application of a cholinergic drug to many parts of the brain induces thirst and ingestion of water in the rat, while an adrenergic drug applied to many of the same loci induces hunger and ingestion of food (*1*). Hernández Peón and his associates, working on the cat, have traced a hypnogenic or "sleep circuit" that is triggered by cholinergic drugs and that can be blocked by application of atropine (a cholinergic antagonist) to parts of the system caudal, but not rostral, to the site of cholinergic stimulation (*2*). Neural systems mediating many of these biological drives appear to follow generally parallel paths through limbic and diencephalic structures. Thus, neural specificity (and separation) of function may be determined by a chemical coding system as well as by contiguity of synaptic connections.

Fisher (*3*, *4*) has postulated that the neural basis for the thirst drive consists of complex alternative and reciprocal pathways of neurons susceptible to activation by cholinergic stimulation. Such a system could increase the duration of the drive by supporting reverberatory firing patterns. Also, alternative neural pathways would be available to insure continued function after injury to certain components of the system. The hypothesis provides one explanation for the fact that lesions in many of the brain structures,