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Base Pairing of Adenine and Uracil Derivatives in the Presence of Water

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Synopsis

The association of chloroform-soluble derivatives of uracil and adenine has been examined in chloroform solution in the presence of dissolved water. Analysis by infrared spectroscopy shows that complex formation still occurs in these conditions, and that the extent of association is substantially unchanged by the presence of water. Evidence is presented for the coexistence of two kinds of base pair (involving, respectively, the C_2 and C_4 carbonyl groups of the pyrimidine) in the solutions, and for some displacement in their relative balance by the added water. The binding of water to the C_2 and C_4 carbonyl groups can be separately observed in both the free uracil derivative and its 1:1 complex with 9-ethyladenine. Little or no competition has been found to occur between the formation of base pairs and binding of water to the bases, as judged by measurements of water solubility in chloroform solutions of the bases individually and in 1:1 mixtures. The evidence suggests that this phenomenon can be largely explained by the formation of double hydrogen bonds by the uracil carbonyl groups. Taken together with recent published observations, the results indicate that hydrogen bonding may make a much greater energetic contribution to conformational stability of biopolymers in aqueous solution than has been supposed.

INTRODUCTION

The reasons for the stability of dispersed double helices of nucleic acids in aqueous media are by no means understood. Tobolsky[1] indeed has suggested that their very occurrence presents a thermodynamic paradox within the framework of polymer chemistry in general. Much effort has therefore gone into attempts to analyze the energetic components of such systems (for a recent review see Leng et al.[2]). In experimental terms, it is known that hydrogen bonds are present between bases on opposite strands of the double helix, in the configuration formulated by Watson and Crick, and the existence of other interactions involving the sugar and phosphate residues has also been postulated. The best characterized properties of the nucleotide units are the vertical stacking interactions, which are generally held to play a dominant role in stabilizing the ordered conformation. These interactions can be ob-

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served between free bases or nucleosides in concentrated aqueous solution, and evidently have a hydrophobic component, for they are eliminated when nonaqueous solvents are introduced and have never been observed in media other than water. By contrast, it has been amply demonstrated[3–6] that suitable derivatives of nucleic acid bases will form hydrogen-bonded pairs in solution in inert solvents, such as carbon tetrachloride and chloroform. The favorable free energy for such an association does not however answer the question of whether hydrogen bonding between the bases stabilizes the nucleic acid helix in aqueous solution. In the presence of a large molar excess of a strong hydrogen-bonding solvent, such as water, the formation of interbase hydrogen bonds will be a competitive process. Clearly the sequestration of a hydrogen-bonding site from the solvent, without the formation of an alternative, i.e., base–base hydrogen bond, would in general be expected to be energetically catastrophic. At the same time, an unfavorable free energy change on replacing a base–water by a base–base hydrogen bond need not prevent a base-paired structure from forming, in view of the existence of other, and overriding, sources of structural stabilization. The free energy of double-helix formation is in fact the small resultant of several large positive and negative contributions. To determine whether in this sense hydrogen bonding between base pairs stabilizes or destabilizes the double helix, it is necessary to evaluate the free energy difference $\Delta F^0_{ass} - \Sigma\Delta F_{-W}$ for the bases in an inert solvent, where ΔF^0_{ass} is the standard free energy of formation of hydrogen-bonded pairs, and the sum comprises the free energies of hydrogen bonding of water to the sites involved in base pairing. We have attempted to assess the effect of water on the base pairing of adenine and uracil by this means.

EXPERIMENTAL

The chloroform-soluble derivatives, 1-cyclohexyluracil, 1-cyclohexyl-5-bromouracil, and 9-ethyladenine, were obtained from Cyclo Chemical Corporation. Deuterochloroform and deuterium oxide were from Koch-Light, and analytical-grade chloroform from British Drug Houses Ltd. The preservative ethanol was removed from chloroform by multiple extractions with water. The resulting material was dried over Drierite molecular sieve and distilled. The product was further dried over phosphorus pentoxide in a desiccator. Chloroform and solutions of bases were saturated with water by isopiestic equilibration, as described by Christian et al.[8,9] For this purpose, Pyrex screw-capped vials, 8 × 3 cm, were constructed, containing an inner vessel in the form of a tube, 1 cm in diameter and 2 cm high, sealed into the bottom of the vial. The chloroform solution (0.5–1 ml) was introduced into the central compartment and water in the outer space. A liquid-tight seal was made by a Teflon gasket under the cap, and the tubes were immersed in a thermostat bath at 25°C and allowed to equilibrate for 24 hr. As ob-

served by Christian et al.[8,9] equilibration is complete in a matter of hours. The chloroform solutions were analyzed for water by gas chromatography.[10] A stainless-steel Poropak Q column (5 × 180 mm) was used in a Perkin-Elmer model KP-8 instrument, operated at a column temperature of 200°C, with nitrogen as carrier gas. The katharometer detector was maintained at 250°C. Determinations were made at a series of sample volumes from 5 to 30 μl, and the water content was determined from the slope of a plot of sample volume against peak area. In some experiments, dry methanol was added to the sample solutions as an internal standard. The reliability of the determinations was checked initially on water-saturated chloroform by Karl-Fischer titration, using the modified Karl-Fischer reagent.[11]

Infrared spectra were measured in a Grubb Parsons "Spectromaster" grating instrument, on both dry and D_2O-equilibrated chloroform-*d* solutions. The bases were first deuterated by twice lyophilizing them from D_2O solution and then drying over phosphorus pentoxide in a desiccator. Calcium fluoride cells of 25- or 75-μm pathlength were used. Because of the contributions of water and D_2O, free and bonded, in the N-H stretching region, observations were made in the 1650–1750-cm^{-1} range, where the primary contributions are from the ring carbonyl groups of the uracil. Whereas water interferes with measurements in this region, D_2O at the concentrations in question is transparent, and was used throughout. The bases were deuterated to avoid complications from deuterium exchange. The association of bases in dry chloroform was also determined from measurements at 3527 cm^{-1} (free NH_2 antisymmetric stretch), using 1-mm and 1-cm silica cells selected for good transmittance.

RESULTS

The infrared spectrum of cyclohexyluracil in chloroform-*d* is considerably changed on introduction of D_2O (Figure 1). In this region, the principal absorption bands arise from the two carbonyl groups of the uracil ring, probably coupled to the N-H group, with some contributions due to the ring double bonds of both bases.[12] In the limit of total elimination of base pairing by the water, the spectrum of the equimolar mixture of the uracil and adenine derivatives, saturated with water, should be that of the sum of the separated components at the same concentration, likewise saturated with water. As Figure 2 shows, this is far from the case. The inference that base pairing substantially persists at the concentrations of water present in these solutions is borne out by a large effect of total base concentration on the spectrum of the water-saturated solution of the mixture.

The total concentration of water in the system was determined by gas chromatography. There is a linear dependence of total water concentration on the concentration of base (Figure 3). Within the limits of our

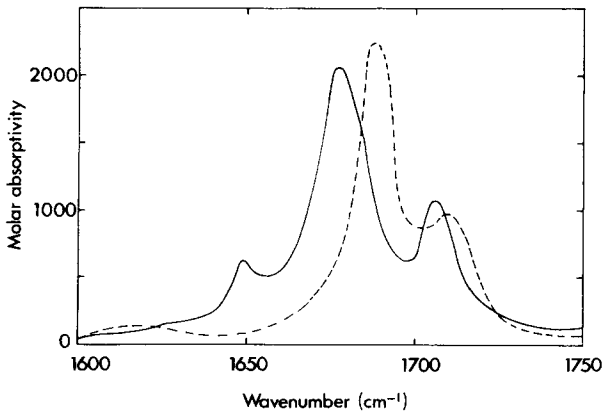


Fig. 1. Infrared spectrum of 1-cyclohexyluracil (deuterated) in chloroform-*d*; dry (broken line) and saturated with D_2O (full line).

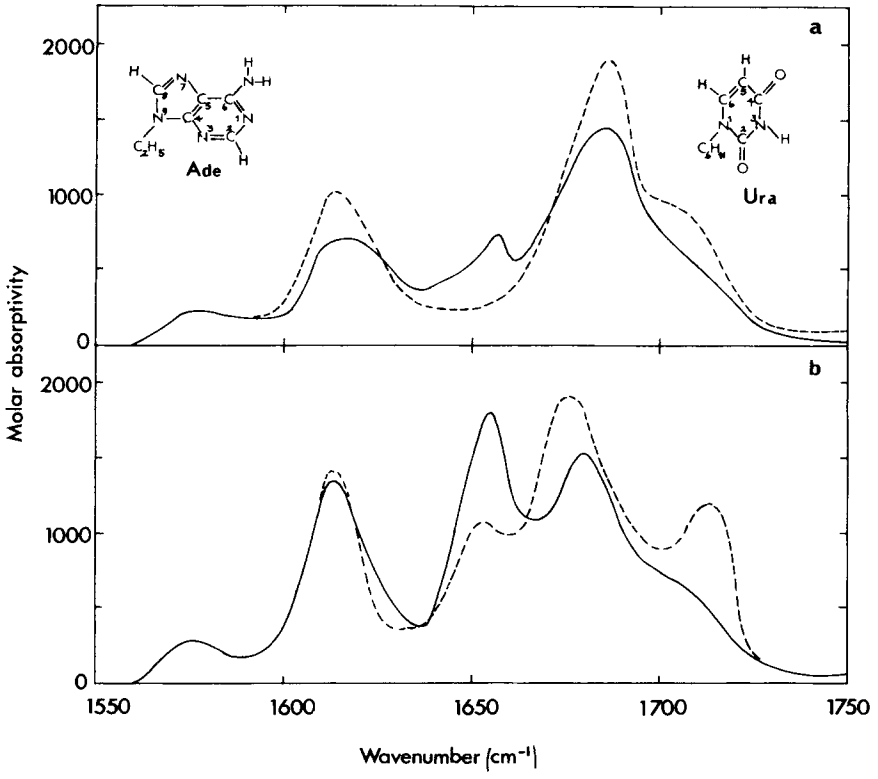


Fig. 2. Effect of association on the infrared spectrum of an equimolar mixture of 1-cyclohexyluracil and 9-ethyladenine (both deuterated). (a) In dry chloroform-*d*. (b) Saturated with D_2O. The broken line refers to the dilute solution, i.e., the sum of the spectra of the separate bases, and the full line to the mixture at 0.1 *M* total base concentration.

experimental error, moreover, the concentration of solubilized water is unchanged on mixing the solutions of the two bases, whereas if base-pairing occurred and if the association constant were similar to that in the dry system,[5] the bases would be in substantial degree associated at a concentration of 0.1 *M*, and most of the additional solubilized water would be displaced, i.e., the solubility of water in the mixture would be diminished. This, of course, will not include any water bound at sites not involved in base pairing, but as will be shown, this is not likely to form a large part of the total. We may carry the analysis a little further, and with the aid of some reasonable assumptions make some esti-

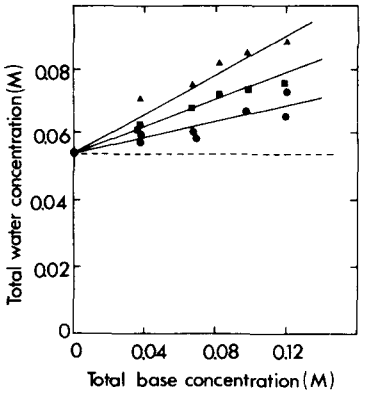


Fig. 3. The solubility of water in chloroform containing 1-cyclohexyluracil (circles), 9-ethyledenine (triangles), and equimolar mixtures of both (squares). The central line corresponds to the average of those for the separate components.

mate of an upper limit of the association constant of the water-bonded bases K^* in the relevant interaction, i.e.:

$$AW_2 + UW_2 \rightleftharpoons A{\cdot}U + 4W$$

where W represents a molecule of water. We assume that Henry's law is obeyed, and that the water-binding equilibria at each hydrogen-bonding site are independent (K_1 and K_2). One may then write for the formal concentration of water in the unmixed solutions of base, B:

$$f_W^0 = [W] + K_1[B][W] + K_2[B][W] + 2K_1K_2[B][W]^2 \equiv [W] + [B]\mu_B$$

with an analogous expression for formal concentration of base, i.e.:

$$f_B = [B] + K_1[B][W] + K_2[B][W] + K_1K_2[B][W]^2 \equiv [B]\lambda_B$$

and with $f_U = f_A$, it may be seen that $\lambda_A[A] = \lambda_U[U]$. Again for the equimolar, interacting mixture:

$$f_W = [W] + \mu_A[A]' + \mu_U[U]'$$
$$f_B = \lambda_A[A]' + \lambda_U[U]' + K_{A\cdot U}[A]'[U]'$$

where the primes denote the concentrations in the mixed solution and $K_{A\cdot U}$ the association constant for Ade and Ura. These equations may be solved. Elimination of the free base concentrations, and cancellation of the terms in μ_A and μ_B gives:

$$\frac{f_W - [W]}{f_W^0 - [W]} = \frac{\lambda_A\lambda_U}{f_B K_{A\cdot U}}\left[\left(1 + \frac{2f_B K_{A\cdot U}}{\lambda_A\lambda_U}\right)^{1/2} - 1\right].$$

With the further assumption that the λ_B are large compared with unity, the term $K_{A\cdot U}/\lambda_A\lambda_U$ leads to $K_{A\cdot U}/K_{A1}K_{A2}K_{U1}K_{U2}$, which is seen to be the equilibrium constant K^*. The left-hand side of this equation is plotted as a function of f_B in Figure 4, together with its experimental values. If we attempt to allow for the water bound at the sites not in-

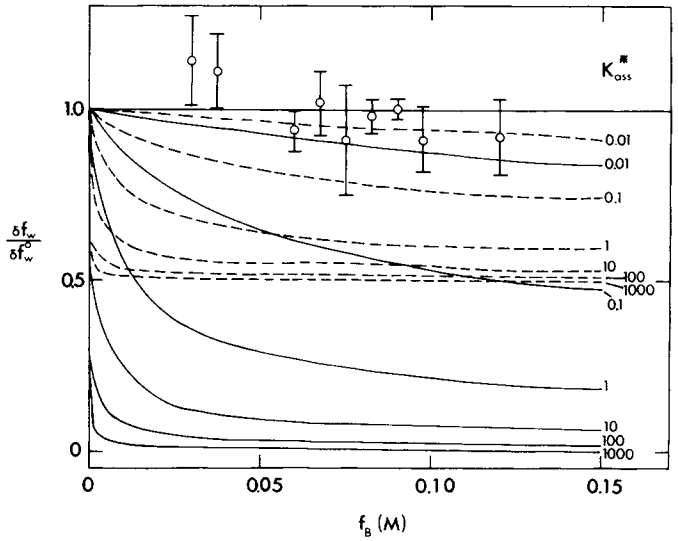


Fig. 4. Calculated curves for the dependence of the function $\delta f_w/\delta f_w^0$, on the concentration of total base in equimolar mixtures in chloroform. These curves express the increase in water solubility occasioned by the presence of the equimolar mixture of the bases, relative to the increase occurring in solutions of the two bases in the absence of base pairing. The full lines are based on the assumption that water can bind only at the base-pairing sites, the broken line on the assumption of four water-binding sites on each base, two of which are implicated in the formation of base pairs. The curves correspond to the indicated values of the association constant for base pairing in competition with water binding. The points are experimental values, showing standard deviations of measurements.

volved in base pairing, and we make the limiting assumption (see below) that this amounts to half the total, which is not subject to displacement on base pairing, we obtain the broken lines in Figure 4. This semiquantitative estimate thus suggests that on a direct interpretation of the water-solubility data, the value of K^* could scarcely correspond to a negative free energy. Qualitatively and at least semiquantitatively, this is apparently irreconcilable with the infrared results. The system, however, is evidently more complex than has been supposed. We have redetermined the association constant for the bases in dry chloroform, working like Kyogoku et al.[5] in the N-H stretching region, but avoiding the assumption implicit in their treatment that the bands of the free and associated forms are completely separable. At 3527 cm^{-1} for example, which is the position of a band arising from free adenine, they assumed that there is no contribution whatever from the dimer, and that the absorbance of the fully associated system at this point would be zero. Inspection of the spectrum however suggests that there could be a substantial overlapping contribution from the dimer band centered at 3404 cm^{-1} at the monomer frequency (and *vice versa*), and our analysis shows that this is in fact the case. We proceed as follows: if the molar absorptivities of the associated and unassociated equimolar mixture (defined in terms of total base concentration) at a given wavelength are ϵ_∞ and ϵ_0, and putting $\epsilon_\infty - \epsilon_0 \equiv \Delta\epsilon_\infty$, $\epsilon - \epsilon_0 \equiv \Delta\epsilon$, where ϵ is the molar absorptivity at concentration $\bar{c}$ (in monomers of total base), the degree of association is $\alpha = \Delta\epsilon/\Delta\epsilon_\infty$. Also the association constant:

$$K = \frac{\alpha}{2\bar{c}(1 - \alpha)^2} = \frac{\Delta\epsilon\Delta\epsilon_\infty}{2\bar{c}(\Delta\epsilon_\infty - \Delta\epsilon)^2}$$

which can be arranged to give

$$\Delta\epsilon = \Delta\epsilon_\infty - \left(\frac{\Delta\epsilon_\infty}{2K}\right)^{1/2}\left(\frac{\Delta\epsilon}{\bar{c}}\right)^{1/2}.$$

Then a plot of $\Delta\epsilon$ against $(\Delta\epsilon/\bar{c})^{1/2}$ should give a straight line, the intercept and slope of which allows the evaluation of $\Delta\epsilon_\infty$ and thus ϵ_∞, and of K. The value of ϵ_0 can be obtained from measurements of the absorbance of the 9-ethyladenine alone at low concentration. Our value for this is 125, which agrees satisfactorily with data of Kyogoku and coworkers[13-16] (though apparently less well with their earlier paper[5]). The extent of competitive self-association in these solutions can be neglected.[5] Excellent linear plots are obtained, and reflect a 1:1 association with $K = 100\ M^{-1}$. The value of ϵ_∞ is 20, not 0 as implicitly assumed in the treatment of Ref. 5. Our attempts to obtain equilibrium constant, using the absorbances measured in the carbonyl stretching region (Figure 2) by the same procedure, gave less satisfactory results, and allowing for an inherently lower precision of measurement, plots of good linearity were not obtained. The change of absorbance at 1655 cm^{-1}

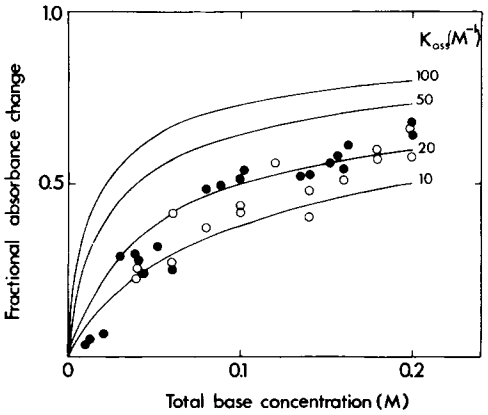


Fig. 5. Fractional change in absorbance at 1655 cm^{-1} with total base concentration in equimolar mixtures of 1-cyclohexyluracil and 9-ethyladenine in chloroform-*d*; dry (open circles) and saturated with D_2O (filled circles). The curves are calculated for the indicated association constants.

with concentration is shown in Figure 5, and best conforms to an equilibrium constant of ~20 M^{-1}. This discrepancy is most simply explained in terms of the (unsurprising) existence of two, energetically essentially degenerate, types of base pair in the system. There are four sterically acceptable arrangements for the A·U pair, one of them the natural[17] Watson–Crick pair, in which it is the C_4 carbonyl group of the pyrimidine that is implicated in the base pairing. In two of the alternative structures base pairing involves the C_2, and in the remaining one the C_4 carbonyl. All four configurations have been observed in mixed crystals of adenine and uracil derivatives.[18] The view that C_2- and C_4-bonded forms are present in solution may in fact be inferred from changes in the spectrum on association of the bases in chloroform (Figure 2a). The carbonyl stretching frequencies at 1708 and 1687 cm^{-1}, which arise, respectively, from the C_2 and C_4 carbonyl groups of the pyrimidine, are both drastically perturbed by the association. We have examined, moreover, mixtures of 9-ethyladenine with the 5-bromo derivative of 1-cyclohexyluracil, in the hope that a bulky substituent in the vicinity of C_4 would change the balance of the base-pairing modes in favor of C_2; this in fact is what occurs in the crystal of the adenosine-5-bromouridine complex.[19] We find that the change in the C_4 carbonyl band in marked contrast to the C_2, does indeed seem to be largely eliminated in the base pair formed by the 5-bromo derivative.

The effect of D_2O on the infrared spectrum of cyclohexyluracil (Figure 1) shows that both carbonyl groups enter into hydrogen bonds. The band at 1650 cm^{-1} must be presumed to represent the D_2O-bound C_4 carbonyl group, the larger band centered at 1677 cm^{-1} a mixture of free C_4 and D_2O-bonded C_2 carbonyl, and that at 1706 cm^{-1} the residual unbonded C_2 carbonyl. In uridine,[20] or the essentially identical random

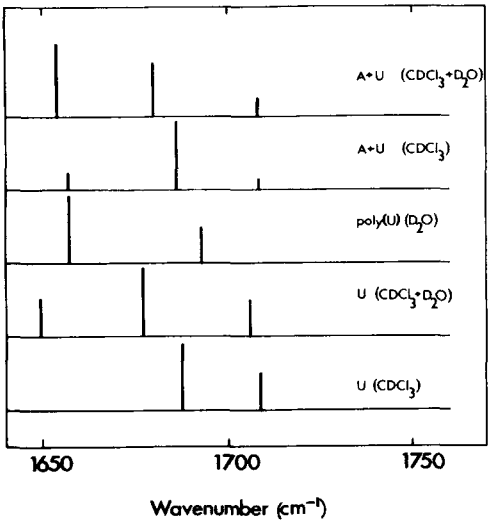


Fig. 6. Frequencies of infrared absorption bands (schematic) in the amide I region of the uracil molecule in free and associated states.

polyuridylic acid,[21] in D_2O solution, the C_2 and C_4 carbonyl peaks appear at 1692 and 1657 cm^{-1}. The displacement relative to wet chloroform must contain a component of solvent shift, but is probably primarily due to the double hydrogen-bonding mode discussed below. The spectra in the carbonyl region of the relevant derivatives are summarized in schematic form in Figure 6. As a check on the assignments, we have followed the effect of D_2O on the spectrum of cyclohexyluracil in acetonitrile solution (Figure 7). This solvent can bond only to the amide N-H group, and the carbonyl bands are left unperturbed, at the same frequency as in chloroform. As D_2O is added, the C_2 and C_4 carbonyl bands both shift to their new positions, characteristic of the D_2O-bonded forms; the free C_2 carbonyl band is first apparent as a shoulder at about 1705 cm^{-1}, and with the diminution in intensity of the adjoining band resolves itself into a peak at 1705 cm^{-1}. The new D_2O-bonded C_2 carbonyl frequency appears as a shoulder at about 1676 cm^{-1} on the low-frequency side of the free C_4 carbonyl band, which lies at 1688 cm^{-1}. As this latter gives place to the D_2O-bonded species at 1653 cm^{-1}, the bonded C_2 gives rise to a maximum at 1674 cm^{-1}. Over a wide range of D_2O concentration, all four species evidently coexist, though only three are ever fully resolved. Ultimately, the free carbonyl frequencies disappear, and the spectrum approaches that seen in pure D_2O solution, allowing for solvent shifts.

Despite the partial implication of both carbonyl groups in hydrogen bonds to D_2O, the extent of pairing of Ade and Ura in deuterochloroform appears to be little affected by added D_2O. Because of the number of species that can exist in this system, it would be hazardous to at-

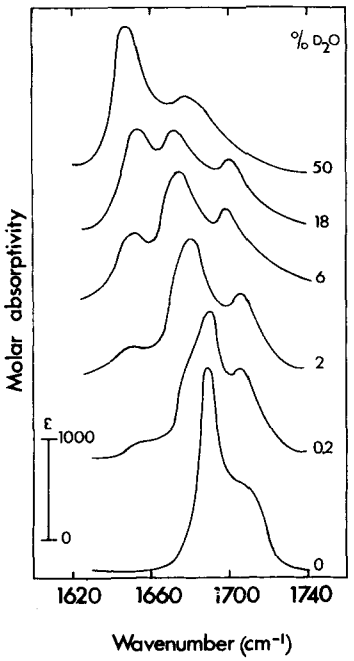


Fig. 7. Infrared spectra of 1-cyclohexyluracil in acetonitrile solution at the indicated concentrations of added D_2O.

tach too much quantitative significance to an apparent A·U association constant, derived as shown in Figure 5, but the results clearly indicate that there is very little difference between the fractional association of the wet and the dry bases at identical total concentrations. However, the introduction of D_2O causes changes in the spectrum of the A·U pairs, which affect both carbonyl groups, with in particular, a large increase in intensity of the band at 1657 cm^{-1}, arising from the hydrogen-bonded C_4 carbonyl group. At the same time, the intensity of the composite band at 1686 cm^{-1}, which contains the hydrogen-bonded C_2 carbonyl frequency, diminishes, and shifts almost to the position associated with the D_2O-bonded C_2 carbonyl group (Figures 6 and 7). This strongly suggests that the D_2O, while not appreciably changing the total extent of base pairing, shifts the balance of populations in favor of a C_4-bonded configuration.

DISCUSSION

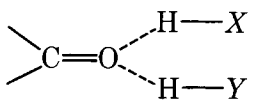
Our results indicate that the system is rather more complex than is at first sight apparent. The association constant in dry solution, determined from the absorbance of the free —NH_2 stretching band is similar to those given by Kyogoku et al.[5] though as noted their treatment involves a doubtful assumption, which may explain the twofold difference

between the values determined at two different wavelengths, (although this difference has since been stated to have vanished[22]). It appears highly probable that two (or conceivably more) energetically practically degenerate types of base pair, implicating, respectively, the C_4 and C_2 carbonyl groups of the pyrimidine, coexist in these solutions. Consequently, an interpretable nominal association constant can be obtained from the concentration-dependent changes of a band arising from the amino group of the 9-ethyladenine. No such result should however, emerge from analyses based on the carbonyl region, since the two uracil carbonyl (amide I) bands are well separated. This is in accord with our observations. We may exclude any important effect of deuteration on the magnitude of the association parameters.[23]

Molecular orbital calculations[24] indicate that the basicities of the two carbonyl groups of uracil are very similar, with point monopoles of -0.55 and -0.53 on the C_2 and C_4 carbonyl oxygens. Moreover, in Coulombic terms the Watson–Crick base pair was only very marginally favored.[24] It is thus reasonable that the binding of water should cause a change in the balance between the types of base pairs present in the solution.

It is obvious (Figure 1) that there is appreciable hydrogen bonding of water to the uracil carbonyl groups in chloroform solution. There is good evidence that the amide N-H group has an extremely low hydrogen-bonding tendency, compared with the carbonyl group[25–27], and one may suppose that the N-H group remains free in the monomeric uracil, even in the presence of water. (This is consistent with the formation of 1:1 complexes of the same derivatives in dimethyl sulphoxide, a solvent that must compete at proton donor sites[28]).

The failure of the A·U interaction to displace water from the hydrogen-bonding sites, and contrariwise the apparently largely unimpaired base pairing in chloroform solutions saturated with water, at first sight appear paradoxical. One possible explanation is that the water may be bound mainly at the hydrogen-bonding sites not implicated in base pairing. In the case of the uracil derivative at least, this may be ruled out on the grounds of the infrared changes (though the basicities of the ring nitrogens in the purine[24] make it quite likely in the 9-ethyladenine). Second, water displaced from the base-pairing sites when the A·U interaction takes place might be rebound to about the same extent at other sites, but this would imply that the basicity or acidity of other donor or acceptor sites would be significantly modified by interactions occurring at the base-pairing groups. This would be energetically an improbable phenomenon. A third and more attractive hypothesis is that the uracil carbonyls are able to form complexes of the type



Such structures are reported to prevail in many interactions of both ketonic and amide carbonyl groups[27,29,30,31] (for review see Ref. 32). Indeed water evidently binds to *N,N*-dimethyl acetamide in this way, with a large shift in the amide I absorption band, and it has been suggested that this is the normal state of the amide (or peptide) group in aqueous solution.[26] It may be seen (Figure 6) that especially the C_4 carbonyl band in uracil undergoes a further shift when it passes from the water-bonded state in chloroform to an aqueous medium. The positions of the bands in the associated A·U systems in the presence of water are presumed then to reflect a double hydrogen-bonding scheme.

As judged by hydrogen-bond lengths in crystals, hydrogen bonds involving water appear to be generally rather strong, compared at least to those between the types of groups involved in base pairing of nucleotides.[33] It might therefore have been expected that in the simplest terms, hydrogen bonding gives rise to a destabilizing contribution to the free energy of nucleic acid double helices in aqueous solution. Whereas the complexity of even the simplest model system is evidently too great to allow the assignments of unique free energy values, we may nevertheless conclude that not merely is the hydrogen-bonding interaction between base and base stronger than that between the bases and water, but that a large molar excess of water would be needed to cause dissociation of the base pairs. (The free energy difference will of course be mitigated by the unitary entropy advantage that accrues from closing the cyclic dimer configuration.) Our observations are consistent with the finding of Binford and Holloway,[34] that neither the association constant nor the enthalpy of association of the adenine and uracil derivatives in chloroform solution are appreciably affected by the presence of a large molar excess of ethanol.

The competitive efficacy of water in relation to hydrogen-bonded interactions has recently been questioned on the basis of calorimetric measurements on thymine in aqueous solution,[35] with the implication that the thermodynamic contribution of the hydrogen bond to the stability of biopolymer structures in aqueous solution should be reassessed. Moreover, it has been inferred from PMR spectra of nucleotide mixtures that hydrogen-bonded association (as opposed to simply stacking interactions) occurs to a perceptible degree in water.[36] Taking into consideration the magnitude of the contribution of the unitary entropy to the free energy of association (some 2 kcal/mol), this striking result suggests that hydrogen bonding may after all be an important determinant of double-helix stability in physiological conditions. It seems justifiable to suggest that the hydrogen bond should perhaps be rehabilitated as an energetically important factor in biopolymer conformation.

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References

1. Tobolsky, A. V. (1964) *J. Phys. Chem.* **68,** 2267–2272.
2. Leng, M., Dourlent, M. & Hélène, C. (1973) in *Physico-Chemical Properties of Nucleic Acids,* Duchesne, J., Ed., Academic, New York, Vol. 3, pp. 19–57.
3. Küchler, E. & Derkosch, J. (1966) *Z. Naturforsch.* **21b,** 209–216.
4. Hamlin, R. M., Lord, R. C. & Rich, A. (1965) *Science* **148,** 1734–1737.
5. Kyogoku, Y., Lord, R. C. & Rich, A. (1967) *J. Amer. Chem. Soc.* **89,** 496–504.
6. Pitha, J., Jones, R. N. & Pithova, P. (1966) *Can. J. Chem.* **44,** 1045–1050.
7. Millar, J. H. & Sobell, H. M. (1967) *J. Mol. Biol.* **24,** 345–350.
8. Christian, S. D., Affsprung, H. E. & Johnson, J. R. (1963) *J. Chem. Soc.* 1896–1898.
9. Christian, S. D., Affsprung, H. E., Johnson, J. R. & Worley, J. D. (1963) *J. Chem. Educ.* **40,** 419–421.
10. Hogan, J. M., Engel, R. A. & Stevenson, H. F. (1970) *Anal. Chem.* **42,** 249–252.
11. Mitchell, J. & Smith, D. M. (1958) *Aquametry,* Interscience, New York, pp. 147 et seqq.
12. Shimanouchi, T., Tsuboi, M. & Kyogoku, Y. (1964) *Advan. Chem. Phys.* **7,** 435–498.
13. Kyogoku, Y., Lord, R. C. & Rich, A. (1969) *Biochim. Biophys. Acta* **179,** 10–17.
14. Kyogoku, Y., Lord, R. C. & Rich, A. (1968) *Nature* **218,** 69–72.
15. Kyogoku, Y., Lord, R. C. & Rich, A. (1966) *Science* **154,** 518–520.
16. Kyogoku, Y. & Yu, B. S. (1969) *Bull. Chem. Soc. Jap.* **42,** 1387–1398.
17. Arnott, S. (1970) *Progr. Biophys. Mol. Biol.* **21,** 265–319.
18. Voet, D. & Rich, A. (1970) *Progr. Nucleic Acid Res.* **10,** 183–265.
19. Haschemeyer, A. E. V. & Sobell, H. M. (1963) *Proc. Nat. Acad. Sci. U.S.* **50,** 872–877.
20. Miles, H. T. (1964) *Proc. Nat. Acad. Sci. U.S.* **51,** 1104–1109.
21. Miles, H. T. & Frazier, J. (1964) *Biochem. Biophys. Res. Commun.* **14,** 90–94.
22. Chen, M. C. & Lord, R. C. (1974) *Biochim. Biophys. Acta.* **340,** 90–94.
23. Boettcher, J. W. & Drago, R. S. (1974), *J. Phys. Chem.* **78,** 429–431.
24. Nash, H. A. & Bradley, D. F. (1966) *J. Chem. Phys.* **45,** 1380–1386.
25. Cannon, C. G. (1955) *Mikrochim. Acta.* **2,** 555–588.
26. Combelas, P., Garrigou-Lagrange, C. & Lascombe, J. (1973) *Biopolymers* **12,** 611–625.
27. Combelas, P., Garrigou-Lagrange, C. & Lascombe, J. (1974) *Biopolymers* **13,** 577–589.
28. Katz, L. & Penman, S. (1966) *J. Mol. Biol.* **15,** 220–231.
29. Morcom, K. W. & Travers, D. N. (1965) *Trans. Faraday Soc.* **61,** 230–234.
30. de Lozé, C., Combelas, P., Bacelon, P. & Garrigou-Lagrange, C. (1972) *J. Chim. Phys.* **69,** 397–403.
31. Combelas, P., Cruege, F., Lascombe, J., Quivoron, C., Rey-Lafon, M. & Sébille, B. (1970) *Spectrochim. Acta* **26A,** 1323–1335.
32. Combelas, P., Garrigou-Lagrange, C. & Lascombe, J. (1970), *Ann. Chim.* **5,** 315–325.
33. Fuller, W. (1959) *J. Phys. Chem.* **63,** 1705–1717.
34. Binford, J. S. & Holloway, D. M. (1968) *J. Mol. Biol.* **31,** 91–99.
35. Alvarez, J. & Biltonen, R. (1973) *Biopolymers* **12,** 1815–1828.
36. Raszka, & M. Kaplan, N. O. (1972) *Proc. Nat. Acad. Sci. U.S.* **69,** 2025–2029.

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