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ADENINE-THYMINE PAIRING IN WATER INDUCED BY AN INTERCALATING AGENT

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Summary : Thymine linked by a $-(CH_2)_3-$ chain to proflavine folds face-to-face with the proflavine in dilute aqueous solution and induces complementary intermolecular face-to-face complexation of a free adenine derivative in the solution.

The interaction of adenine with thymine and of cytosine with guanine - base pairing - is an important structural feature of deoxyribonucleic acids, yet it does not seem to occur in aqueous solutions of complementary bases[1]. The matrix of the double helix structure of DNA with its stacking interactions of successive layers of bases makes such pairing thermodynamically favourable.

When one of the above bases is attached by a short chain as in 1 to a molecule known to intercalate in DNA the molecule exists[2a] to 100% in a folded conformation 2 in dilute aqueous solution. Thus the stacking interaction in DNA is induced in this fragment.

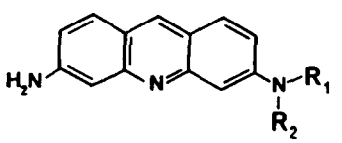
We now report that when the intercalant is proflavine 3, not only does the heterodimeric molecule Pf-C_3-Thy 4 or Pf-C_3-Ade 8 exist to 100 % in a folded conformation, but more interestingly a free molecule of the complementary derivative 5 present in solution will now complex with the intercalant-base molecule 4 as in 7. The intercalant induces pairing of complementary bases.

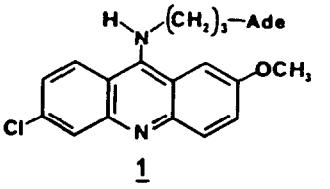
Proflavine 3 is a well-known intercalating agent[3] which, as diagram 9 emphasizes and x-ray studies[4] confirm, may interact comfortably with both of a pair of complementary bases. Linking proflavine to either base by a three-carbon chain (to give Pf-C_3-Ade 8 or Pf-C_3-Thy 4)[5] retains the geometry of the interaction in the folded complex, 10, and should therefore enhance its stability.

Model compounds 4 (Pf-C_3-Thy) and 8 (Pf-C_3-Ade) were prepared from a common precursor, the N-(3-bromopropyl), N-tosyl,3,6-diaminoacridine 11 obtained by treatment (CO_3K_2-DMF)

of N-tosyl,3,6-diaminoacridine 12 with 1,3-dibromopropane. Alkylation of 11 with sodium adenylate in DMF followed by acidic treatment (H_2SO_4-AcOH) afforded model compound 8. Alkylation with silylated thymine (sulfolane, 80°) followed by acidic deprotection resulted in the formation of model 4. Reference compound 6 (Pf-C_2) was obtained similarly by ethylation of 12 and deprotection.

When a solution of Pf-C_2 6 dilute enough (2.5×10^{-5}M) to minimize self-aggregation[6] (c.a. 2.7 % dimer) is treated with increasing concentrations of Ade-C_3 5 (from 1.5×10^{-3}M to 7.8×10^{-3}M) all proflavine protons are slightly shifted to high field, which indicates[7,8] a weak interaction, association constant K=80±5 M^{-1}. That there is no specific face-to-face association is confirmed by the absence of significant change in the UV behaviour of proflavine Pf-C_2 with large excesses of either Ade-C_3 or Thy-C_3[2a].





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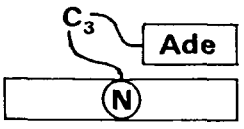
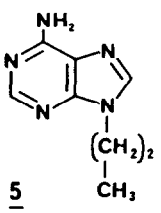


2

3 : $R_1 = R_2 = H$
4 : $R_1 = H$; $R_2 = (CH_2)_2CH_2$-Thy : Pf-C_3-Thy
6 : $R_1 = H$; $R_2 = CH_2CH_3$: Pf-C_2
8 : $R_1 = H$; $R_2 = (CH_2)_2CH_2$-Ade : Pf-C_3-Ade
11 : R_1 = Ts ; $R_2 = (CH_2)_2CH_2Br$
12 : R_1 = Ts ; $R_2 = H$



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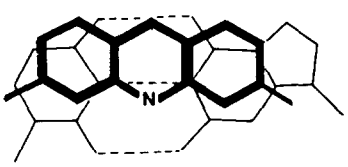

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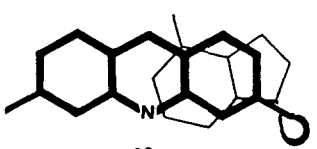

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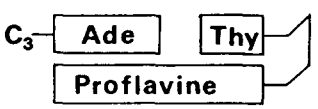

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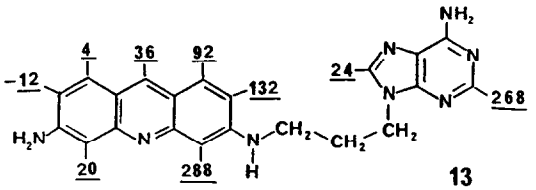
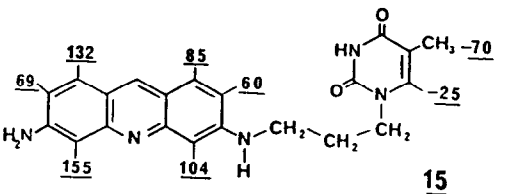
When the base and proflavine are linked together however, as in 8 (Pf-C_3-Ade) and 4 (Pf-C_3-Thy), different behaviour is observed. The UV hypochromic effects, 11% for Pf-C_3-Ade and 7% for Pf-C_3-Thy, are temperature independent up to 70°C, indicating that effectively none of the unfolded form is present even at the high temperature[9]. NMR measurements extrapolated to infinite dilution[10] and set against these for reference compounds, see 13 and 14, show large specific effects, more striking for Pf-C_3-Ade since the ring-current is stronger in adenine than in thymine[11]. Those parts of either the proflavine or the base molecule near to the face-to-face interaction show the largest effects, in accordance with the expected ring-ring stacking geometry[12] of the complex as indicated in 10.

When now the model Pf-C_3-Thy 4 (2.5×10^{-5}M) is treated with increasing concentrations of the complementary base derivative Ade-C_3 5 (from 9×10^{-4} to 5.1×10^{-3}M), changes are observed in the shifts of all protons, see figure 1. The proflavine signals exhibit upfield shifts,





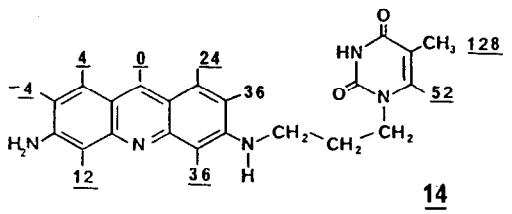


Figure 1 : Changes in ^1H chemical shift (extrapolated to infinite dilution in D_2O, pD 5.5, 20°C. + = upfield shift, Hz at 400MHz). 13 : Differences between Pf-C_3-Ade and Pf-C_2 + Ade-C_3 indicating adenine folded over the right side of the proflavine ring. 14 : Differences between Pf-C_3-Thy and Pf-C_2 + Thy-C_3 indicating thymine folded over the proflavine ring. 15 : Differences between Pf-C_3-Thy + Ade-C_3 and Pf-C_3-Thy indicating that Ade-C_3 associates in a face-to-face fashion with the proflavine ring when thymine is already folded over the proflavine plane[8].

while the thymine H-6 and CH_3 protons are slightly deshielded. The association constant $K=300\pm5\ M^{-1}$ calculated[8] for Pf-C_3-Thy + Ade-C_3 shows a more than threefold increase of the interaction as compared to $K=80\pm5M^{-1}$ determined for Pf-C_2 + Ade-C_3. In 15 all proflavine protons are shielded and the effect is more pronounced on the "left" part of the molecule onto which Ade-C_3 is able to stack. The effect of the proflavine on the thymine is reduced in the presence of Ade-C_3 suggesting that the thymine is slightly displaced to the "right" (see scheme 7).

As a conclusion two points emerge 1/ the intercalating drug proflavine stacks intramolecularly with adenine and thymine in the models 2/ intermolecular interaction between the proflavine nucleus and Ade-C_3 is strongly enhanced by the presence of the covalently linked complementary base in the model. This leads us to conclude that when 4 and 5 are mixed, there is cooperation between adenine and thymine in the two-fold binding process. This suggests that base pairing takes place. There is nothing in our evidence to indicate that hydrogen bonding is involved, but such an interaction seems an attractive explanation for a structure like 7.

Low solubility of guanine derivatives and the low diamagnetic shielding of thymine and cytosine derivatives make experiments with other bases inconclusive.

All results are quite in agreement with the well known observation that intercalators stabilize the structure of DNA, as indicated notably by the increase of the melting temperature T_m[3b,4].

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(1) For general review, see : Saenger, W. in "Principles of Nucleic Acid Structure" ; Cantor, C.R., Ed. ; Springer-Verlag : New-York, **1984** ; pp 116-158.

(2) (a) Bolte, J.; Demuynck, C.; Lhomme, M.F.; Lhomme, J.; Barbet, J.; Roques, B.P. J. Am. Chem. Soc. **1982**, 104, 760-765. (b) Bolte, J.; Demuynck, C.; Lhomme, J.; Fournie-Zaluski, M.C.; Roques, B.P. Biochemistry **1979**, 18, 4928-4935.

(3) For review, see : (a) Albert A. "The Acridines"; Edward Arnold : London, **1966** ; pp 493-503. (b) Wilson, W.D.; Jones, R.L. in "Intercalation Chemistry"; Whittingham, M.S.; Jacobson, A.J. Eds.; Acad. Press : New York, **1982** ; pp 445-501.

(4) Neidle, S. "Topics in Nucleic Acid Structure", Neidle, S. Ed.; Mac Millan Pub. : London, 1981 ; Vol 1, **1981** ; pp 177-196. Aggarwal, A.K.; Neidle, S. Nucl. Acids Res., **1985**, 13, 5671-5684.

(5) Abbreviations used : Ade for aden-9-yl ; Thy for thym-1-yl, Pf for 6-amino-3-(-yl)aminoacridine, C_2 for ethyl, and C_3 for n-propyl. All new substances were characterized by full spectra and elemental analysis. Compound 4 had : mp 208-212° ; ^{1}H-NMR (60MHz, DMSO d_6) 1.65 (3H, s, ThyC$\underline{H}_3$), 1.90 (2H, m, C$\underline{H}_2$), 3.25 (2H, t, Pf-C$\underline{H}_2$), 3.85 (2H, t, Thy-C$\underline{H}_2$), 6.60 (1H, s, PfC$_4\underline{H}$), 6.70 (1H, s, PfC$_5\underline{H}$), 6.75 (2H, 2d, PfC$_2\underline{H}$, C$_7\underline{H}$), 6.80 (2H, s, PfN$\underline{H}_2$), 7.60 (2H, 2d, PfC$_1\underline{H}$, C$_8\underline{H}$),7.80 (1H, s, Thy$\underline{H}_6$), 8.55 (1H, s, PfC$_9\underline{H}$). Microanalysis : C, 56.37 ; H, 5.60 ; N, 15.34 ; O, 13.76. $C_{21}H_{21}N_5O_2$, HCl, $2H_2O$ requires : C, 56.31 ; H, 5.85 ; N, 15.63 ; O, 14.28.
Compound 8 had : mp 226-231° ; ^{1}H-NMR (60MHz, DMSO d_6) 2.20 (2H, m, C$\underline{H}_2$), 3.15 (2H, t, Pf-C$\underline{H}_2$), 4.10 (2H, t, Ade-CH_2), 5.75 (2H, s, PfN$\underline{H}_2$), 6.65 (1H, s, PfC$_4\underline{H}$), 6.80 (1H, s, PfC$_5\underline{H}$), 6.90 (2H, 2d, PfC$_2\underline{H}$, C$_7\underline{H}$), 7.00 (2H, s, AdeN$\underline{H}_2$), 7.65 (2H, 2d, PfC$_1\underline{H}$, C$_8\underline{H}$), 8.15 (1H, s, AdeC$_2\underline{H}$ or C$_8\underline{H}$), 8.25 (1H, s, AdeC$_8\underline{H}$ or C$_2\underline{H}$), 8.40 (1H, s, PfC$_9\underline{H}$). Microanalysis : C, 49.67 ; H, 5.56 ; N, 21.99 ; O, 9.28. $C_{21}H_{20}N_8$, 2HCl, $3H_2O$ requires : C, 49.32 ; H, 5.51 ; N, 21.91 ; O, 9.38.

(6) This % is calculated from the auto-association constant for Pf-C_2 K=1100 M^{-1}. This is the mean value calculated[8] for the different protons from chemical shifts variations -deshielding- observed in the concentration range $2x10^{-3}$-$2x10^{-5}$M. Ade-C_3 is much less prone to aggregation (2).

(7) ^{1}H NMR spectra were recorded at 400MHz . Aqueous solutions were made in a deuteroacetate buffer (pD 5.5) to ensure protonation of the proflavine ring.

(8) Dimicoli, J.L.; Helene, C. J. Am. Chem. Soc. **1973**, 95, 1036-1044.

(9) See Leonard, N.J. Acc. Chem. Res. **1979**, 12, 423-429 for use and significance of hypochromic effects in the study of ring-ring stacking interactions. See ref 2a for discussion of the temperature dependance of the percent hypochromism %H.

10) Extrapolated values obtained from measurements run in the ranges : $1.3x10^{-3}$-$6x10^{-5}$M for Pf-C_3-Ade and $5x10^{-4}$-$1.5x10^{-5}$M for Pf-C_3-Thy.

(11) (a) Giessner-Prettre, C.; Pullman, B. Biochem. Biophys. Res. Commun. **1976**, 70, 578-581. (b) Giessner-Prettre, C.; Pullman, B. C.R. Acad. Sci., Ser. D **1976**, 283, 675-677.

(12) For Pf-C_3-Ade in particular, correct fit is observed between extrapolated chemical shifts differences and the values calculated from the isoshielding curves of adenine[11a] and proflavine[11b] in the geometry shown in 10.

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