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Stacking self-association of pyrimidine nucleosides and of cytosines: effects of methylation and thiolation[1]

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ABSTRACT

Stacking self-association equilibria in aqueous solutions of m^3uridine, $m_4^{3,2',3',5'}$uridine, 2'-deoxyuridine, $m_3^{1,4,4}$cytosine, $m_4^{1,4,4,5}$cytosine, s^2cytidine and s^4thymidine were studied at various temperatures by vapour-pressure osmometry. Equilibrium constants K_{st}'s were computed on the assumption of the isodesmic model of self-association. Enthalpies of association were also obtained from the temperature dependence of K_{st} according to the van't Hoff equation. Analysis of the equilibrium and thermodynamic parameters demonstrated involvement of hydrophobic interactions in the stabilization of complexes of tetramethyluridine. Dipole-induced dipole interactions seem to predominate in the formation of s^2C, s^4T and of both dimethylaminocytosine complexes.

INTRODUCTION

In recent years continuing interest is noted in the self-association of nucleic acid bases, nucleosides and nucleotides in aqueous solutions[2] owing to the as yet not well understood nature of the physical forces responsible for stacking of purine and pyrimidine base pairs. In the light of the so far accumulated experimental data, there remains little doubt that electrostatic dipole-induced dipole forces contribute largely to the stacking energy in purine and purine-pyrimidine systems[2,3,4]. The formation of such electrostatically stabilized complexes is accompanied by negative enthalpy and entropy

changes[2]. Our recent osmometric studies[5] have confirmed this point also in respect to the self-association of diketopyrimidines, particularly of those bearing polar substituents. At the same time, in the formation of complexes of N- and C- substituted with two or more alkyl groups uracil derivatives, simultaneous involvement of classical hydrophobic interactions[6], characterized by positive enthalpy and entropy changes, has been demonstrated by us[5]. It appeared thus of great interest to extend these studies to some methylated uridine and cytosine derivatives in order to examine the effect of methyl substitution on their stacking.

Replacement of keto by more polarizable thioketo group(s) in uridine has been shown to enhance stacking interactions in aqueous solutions of free nucleosides[7] as well as thermal stability of the helical polynucleotides in which the 2-thioketo group comes in close contact with the neighbouring heterocyclic ring[8]. To complete our studies on stacking of thioketopyrimidine nucleosides we now measured association equilibria of 4-thiothymidine and 2-thiocytidine. The knowledge of their stacking affinities appears of particular interest in connection with recent investigations on the melting properties of poly(s^2C) helix[9], poly(I) : poly(s^2C)[10], poly[r(G-s^2C)][11], and poly[d(A-s^4T)][12].

MATERIALS AND METHODS

$m_4^{1,4,4,5}$Cyt was synthesized according to Kulikowski and Shugar[13], but obtained in crystalline form, m.p.111-112^{0}C(uncor.) in contrast to the oil reported by these authors. s^2C and s^4T were received from Dr K.H. Scheit (Max-Planck-Institut f.Biophysikalische Chemie, Göttingen). $m_4^{3,2';3';5'}$U was prepared by methylation of uridine with methyl iodide in the presence of sodium hydride, under the conditions described by Ponpipom

and Hanessian[14] for 2;3'-O-benzylidene uridine. Colourless crystals were obtained from ethanol: m.p.97-98.5^{0}C, elemental analysis : calc.: C 51.97%, H 6.72%, N 9.33%, found: C 51.91%, H 6.71%, N 9.21%. The remaining compounds were obtained by routine methods[15] and thoroughly purified.

Determinations of osmotic coefficients at various solute concentrations were performed with a Knauer vapour-pressure osmometer by procedures used earlier[5,7].

RESULTS AND DISCUSSION

The vapour-pressure osmometric data were analysed under the assumption of the isodesmic model of multistep association ($K_1=K_2=\ldots=K_n=K_{st}$), as has been described previously[5]. The values of stacking association equilibrium constants (K_{st}) in water at various temperatures derived thereof are presented in the Table together with corresponding standard enthalpies ΔH^0,

TABLE

compound	K_{st} [molal^{-1}]					ΔH^0 [kcal/mole]	ΔS^0 [eu]
	25^{0}C	35^{0}C	45^{0}C	50^{0}C	60^{0}C		
uridine[a]	0.64±0.05		0.53±0.03		0.44±0.02	-2.1±0.2	-7.8±0.7
2'-deoxyuridine	0.74±0.03						
m^3uridine	0.65±0.06		0.48±0.03		0.40±0.03	-2.7±0.1	-10.1±0.2
$m_4^{3,2';3';5'}$uridine	0.66±0.08	0.75±0.12	0.93±0.04		0.90±0.10	+3.2±0.5[b]	+9.9±1.7
$m_3^{1,4,4}$cytosine	1.8±0.3		1.5±0.2		1.0±0.1	-2.8±0.7	-8.2±1.7
$m_4^{1,4,4,5}$cytosine	(~4)[c]		2.2±0.3	1.9±0.2	1.5±0.1	-5.4±0.1	-15.3±1.5
cytidine[a]	0.95±0.08	0.83±0.08	0.77±0.09		0.56±0.04	-2.9±0.4	-10.0±3.0
s^2cytidine	3.7±1.3	2.1±1.1	2.0±0.7		1.7±0.4	-4.0±1.0	-10.6±3.0
thymidine[a]	1.03±0.09	0.81±0.06	0.74±0.07		0.64±0.05	-2.6±0.4	-8.7±1.2
s^4thymidine	4.8±0.8						

a) from ref.5; b) obtained from van't Hoff plot at 25-45^{0}C ; c) exstrapolated value

obtained from van't Hoff plots, and standard entropies ΔS^0 calculated accordingly.

The self-association data for uridine, thymidine and cytidine (see Table) taken from our recent VPO studies[5] are in good agreement with the results of previous osmometric[2] investigations.

Inspection of the data for U and m^3U shows that substitution of the CH_3 group at the N(3) atom in uridine does not exert any influence on the K_{st} value, but seems to increase the negative enthalpy and entropy of association by about 30 per cent. Such an enthalpy-entropy compensation is considered[16] to reflect changes in solute-solvent and solvent-solvent interactions in the solvation shell in aqueous solutions.

The differences in the equilibrium and thermodynamic parameters of self-association of the nucleoside m^3U (see Table) and of its free base m^3Ura ($K_{st}^{25^0C} = 1.13\ M^{-1}$, $\Delta H^0 = -4.9$ kcal.mole^{-1}, $\Delta S^0 = -16.2$ eu)[5] require some comment. The latter are considered as characteristic for a predominant involvement of dipole-induced dipole interactions in the stabilization of stacked complexes because of the high negative enthalpy and entropy values. The smaller K_{st} value and less negative thermodynamic parameters of m^3U, as compared to those of the model m^3Ura, are most probably due to the fact that the number of mutual geometrical orientations available in the process of vertical base-base association of m^3Ura is strongly limited by the presence of the ribose moiety. Configurations with lowest negative enthalpies and entropies are apparently excluded.

Methylation of all three hydroxyl groups of the ribose moiety produces a strong effect on the thermodynamics of association, though at room

temperature there is hardly any difference noted between K_{st} values for m^3U and $m_4^{3,2',3',5'}U$. Here too a profound enthalpy-entropy compensation effect, but of the opposite sign than that observed for the U and m^3U pair, is clearly responsible for the apparent similarity of equilibrium data at room temperature. A similar phenomenon has been noted for the homologous series of alkyl-uracils[5]. Contrary to the observed inverse dependence of K_{st} on temperature, resulting in negative enthalpy of uridine and m^3U, association of $m_4^{3,2',3',5'}U$ increases with the rise of temperature up to about 45^0C and then remains approximately constant up to the upper limit of the temperatures studied, i.e. 60^0C. The van't Hoff plot of K_{st} is not linear (see Fig.1.). From its initial slope within the temperature interval $25-45^0C$ positive enthalpy of association could be estimated, and consequently also positive entropy of this process (see Table). In the light of our previous osmometric studies on association of alkylated uracil derivatives[5], the positive sign of

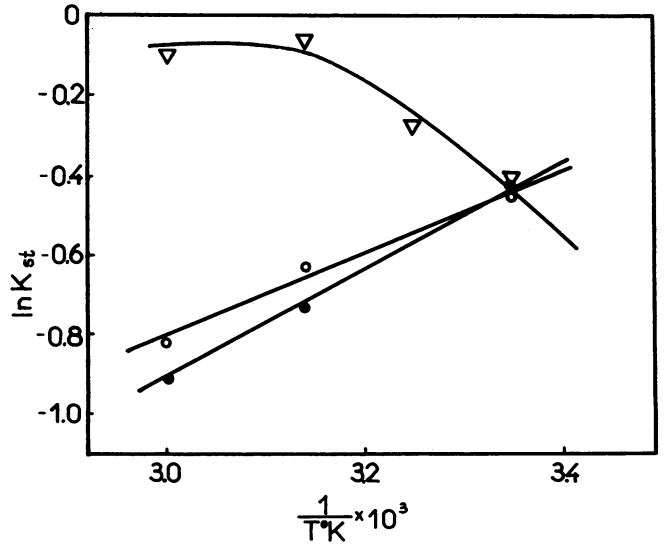

Fig.1. van't Hoff plots of stacking equilibrium constants: o-o-o-uridine, ●-●-●-m^3uridine, ∇-∇-∇-$m_4^{3,2',3',5'}$uridine.

both thermodynamic parameters and variation with the temperature of ΔH^0 are consistent with the predominant contribution of hydrophobic interactions between methyl groups to the stabilization of aggregates. Examination, with the help of CPK models, of the possible mutual orientations of the two nucleoside molecules in associates led us to the conclusion that among the number of configurations involving adherence of 2';3';5' methoxy groups such an arrangement is also possible in which both base-base stacking with carbonyl oxygen overlapping the ring[3] and $CH_3 \ldots CH_3$ contacts between methylated ribosyl moieties are at maximum. The latter configuration seems to be most stable in water.

According to the procedure used previously[5] in derivation of the thermodynamic parameters describing hydrphobic interactions in the association of $m_2^{1,3}e^5$Ura, we have calculated these parameters also for the $m_4^{3,2';3';5'}U$ self-association. The free energies of association at various temperatures are treated as the sum of free energies of hydrophobic (hph) and electrostatic interactions. The electrostatic contribution is assumed to be represented by the thermodynamics of the m^3U association. The free energies of hydrophobic interactions thus obtained are: $\Delta G^T_{hph} = \Delta G^T_{m_4^{3,2';3';5'}U} - \Delta G^T_{m^3U}$ and fit excellently (with standard deviation 0.96 per cent) the function:

$$\Delta G_{hph} = 35613 - 213\ T + 0.313\ T^2 \qquad /1/$$

The remaining, temperature-dependent, thermodynamic parameters derived from /1/ at 298K assume the following values:

$\Delta H_{hph} = 7.8$ kcal.mole^{-1}

$\Delta S_{hph} = 26.4$ eu

$(\Delta c_p)_{hph} = -186$ cal.mole^{-1}.deg^{-1}

Most probably they reflect the formation of 3 to 4 hydrophobic contacts between pairs of nucleoside molecules. When this is taken into account, a reasonable agreement as to the order of magnitude is obtained between our present, former[5] and predicted data of Nemethy and Scheraga[6] and of Ben-Naim and Yaacobi[17] concerning thermodynamics of "hydrophobic bond" formation between two adhering CH_3 groups. It is of great interest in this connection, that the calorimetrically determined[18] (for the first time for heterocyclic systems) excess heat capacities Δc_{p2} of dilute aqueous solutions of caffeine (+ 160 cal.mole^{-1}.deg^{-1}) and theophylline (+ 140 cal.mole^{-1}.deg^{-1}) are of the same magnitude but of opposite sign. This may be taken as an indirect argument in support of the notion that hydrophobic interactions are connected with a partial reversal of hydrophobic hydration of apolar molecules (groups)[6,19].

A minor chemical modification of the sugar moiety seems to have but little effect on the association of nucleosides and this is considered as proof of stacking as a mode of base-base interactions[2]. The association equilibrium constant obtained for 2'-deoxyuridine (see Table) seems to be in accord with this point of view. Its slightly higher value, as compared to that of uridine, may be attributed to a changed state of hydration around the molecule caused by the lack of 2'-hydroxyl group. It is obvious, that without the knowledge of the other thermodynamic parameters of association, this point cannot be further discussed.

It is known from previous studies that the $-NH_2$ group increases the stacking ability of both purines and pyrimidines[2,4,5], cf. for instance the data for uridine and cytidine (see Table). Its replacement by the $-N(CH_3)_2$

group in adenosine brings about a further large increase in the stacking association constant[20]. These effects can be undoubtly attributed to a higher polarizability of the derivatives bearing strongly electron-donating substituents.

At first glance, the thermodynamics of association of both cytosine derivatives studied: $m_3^{1,4,4}Cyt$ and $m_4^{1,4,4,5}Cyt$ (see Table) seems to reflect predominant involvement of electrostatic interactions in the formation of stacked complexes, since respective enthalpy and entropy changes are clearly negative. However, comparison of the equilibrium and thermodynamic data for association of m^1Ura ($K_{st}^{25^0C} = 0.83\ M^{-1}$, $\Delta H^0 = -5.2$ kcal.mole^{-1}, $\Delta S^0 = -17.8$ eu)[5] with that of $m_3^{1,4,4}Cyt$ shows that a higher stacking association constant for the latter compound is accompanied by an about twofold smaller enthalpy and entropy of association. This can be taken as indirect evidence of partial concealment of the negative and positive contribution to the thermodynamic functions, i.e. for "hidden" hydrophobic interaction. Unfortunately, because of the too low solubility of $m_4^{1,4,4,5}Cyt$ in water (about 0.024 M.l^{-1} at 25^{0}C) we were unable to measure association equilibria at temperatures below 45^{0}C, at which hydrophobic interactions are most likely to appear. If we assume that like in the case of $m_3^{1,4,4}Cyt$, the enthalpy of association of $m_4^{1,4,4,5}Cyt$ seems to be temperature-independent within the range of temperatures 25-60^{0}C, then the about twofold increase in K_{st} and both thermodynamic parameters upon C(5) substitution with the CH_3 group would indicate an unexpectedly strong effect of this group, opposite to that observed between the m^1Ura and m^1Thy pair[5]. This, of course, does not seem very probable. An explanation may be thus sought in the nonplanar

conformation of the $-N(CH_3)_2$ group in $m_4^{1,4,4,5}Cyt$ [21,22] forced by steri-cal hindrance of the C(5)-CH_3 group. This can affect the preferred geometry of association as well as polarizability of the ring and polarizing power of the exocyclic amino group lone electron pair.

At present, reconciliation of experimental data with a molecular model based on the above premisses seems rather difficult. Under these circumstances further studies on self-association of better soluble cytidine, 5-methylcytidine and their derivatives bearing monomethyl- and dimethylamino groups might shed more light on the nature of the unique effect of the C(5)-CH_3 group, known to bring about thermal stability in synthetic double helical polynucleotide complexes[23] and in DNA containing m^5Cyt [24].

Stacking association constants of s^4T and s^2C appear about four-fold higher than that of their respective keto analogues (see Table). The same effect was observed by us previously[7] for a series of thiouridines (s^4U, s^2U, s^2s^4U) and interpreted, solely on the basis of equilibrium data, as a result of higher polarizability of thioketo group. Thermodynamic parameters of s^2C association clearly indicate that it is the more negative enthalpy of association, as compared with cytidine, which drives the equilibrium toward stacks formation.

There is thus now convincing evidence from VPO studies on self-association of free pyrimidine nucleosides that each substitution of a thioketo- for a keto- group, i.e.2-, 4- and 2,4- brings about a large increase in the stacking affinity of pyrimidine bases[7] (see also Table). This effect does not manifest itself by an increased melting temperature T_m of all respective helical polynucleotides and of their complexes with complementary purine po-

lynucleotides. The increase of T_m's is observed only for polynucleotides containing a 2-thioketo group bearing uracil or cytosine residues, i.e. poly(s^2U) , poly(s^2s^4U) , poly[r(A-s^2U). r(A-s^2U)], poly(s^2C).poly(I) and poly (s^2C-G) [10,11,25]. The thermal stability of polynucleotides bearing a 4-thioketo group remains but little affected, as in the case of poly(s^4U) and poly(s^4U) .poly(A) [8], or even decreases markedly e.g. poly[r(A-s^4U). r(A-s^4U)][8] and poly[d(A-s^4T)][12]. These differences between both series of polynucleotides must be due to their different packing patterns. Poly(s^2U) packing pattern[8] is characterized by close contact between sulphur and N(1) nitrogen atoms of adjacent bases in the α-chain of the asymmetric helix allowing thus for strong interactions of dipole-induced dipole type. In the polynucleotides containing 4-thiopyrimidines the sulphur atom of the 4-thioketo group engaged in a relatively weak N-H...S=C hydrogen bond[26] does not form such a close contact with an adjacent base. There is one exception in the 2-thioketo series, i.e. poly(s^2C) which in half-protonated form melts at a T_m value by 36^0C lower than that of poly(C) under identical conditions[9]. It is known, however, that ionic forms of bases do not stack[27] so the poly(C) and poly(s^2C) helices must be stabilized by hydrogen bonds of a strong charge-transfer character, N^+-H...O and N^+-H...S, weaker in the latter case.

The availability of the relative stacking affinities of thio-pyrimidines may also prove useful in the elucidation of role played by these bases in folding of tRNA[28].

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