FLUORESCENCE STUDIES ON P^1,P^3-DINUCLEOSIDE TRIPHOSPHATES RELATED TO mRNA *cap*: ACIDITY AND INTRAMOLECULAR STACKING

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The fluorescence intensity of several P^1,P^3-dinucleoside triphosphates related to mRNA *cap*-structure has been measured as a function of pH and temperature. The extent of intramolecular stacking has been estimated by comparing the intensities obtained with dinucleoside triphosphates to those of the corresponding monomers. The pK_a values of 7-alkylguanine moieties in both dimeric and monomeric compounds have been determined by fluorescence and absorption spectroscopy.

A characteristic *cap*-structure, m7G(5′)ppp(5′)N, which constitutes the 5′-end of eukaryotic mRNA's and most viral templates, has been established to play an important role in initiation of protein translation[1,2]. Detailed knowledge of chemical properties of this *cap*-structure itself is a prerequisite for the understanding of this process at molecular level. For example, a factor that largely determines the conformation of *cap*, and may thus affect its chemical behaviour, is intramolecular base-stacking of the 7-methylguanine residue with the adjacent nucleobase[3,4]. The present work is aimed to elucidate the strength of this interaction and to clarify the effects that structural changes in the 7-alkylguanine moiety exert on the thermodynamics of intramolecular base-stacking. Furthermore, the influence of base-stacking on the acidity of the 7-alkylguanine ring, has been considered.

RESULTS AND DISCUSSION

Table I records the fluorescence and absorption spectral properties of the dinucleoside triphoshates (*Ia* – *Va*) and nucleoside monophosphates (*Ib* – *Vb*) studied. It is noteworthy that the spectral properties of *Va* and *Vb* strongly differ from those of the other compounds. As seen, deprotonation of N1 of 7-alkylguanine ring results

TABLE I

Spectral properties and pK_a values of P^1,P^3-dinucleoside triphosphates and their monomeric counterparts

Compound	Absorption λ_{max} [a]		Fluorescence λ_{max}		Fluorescence I_{rel}		pK_a	
	pH 5·1	pH 8·9	pH 5·1	pH 8·9	pH 5·1	pH 8·9	*b*	*c*
Ia	256(232)	254(244)	382	410	72	29	7·35(4)	7·34(4)
IIa	255(229)	254(238)	380	412	32	18	7·43(5)	7·39(4)
IIIa	257(233)	255(243)	386	416	51	20	7·28(4)	7·30(4)
IVa	256(232)	254(244)	380	425	24	22	7·54(5)	7·53(8)
Va	259(235)	244	395	435	160	42	7·46(4)	7·42(4)
Ib	258(232)	281(243)	380	410	100	33	7·24(3)	7·21(3)
IIb	257(231)	282(243)	380	411	67	31	7·31(3)	7·27(3)
IIIb	259(235)	285(247)	386	415	59	21	7·22(3)	7·23(3)
IVb	261(234)	292(272)	380	425	76	54	7·41(3)	7·38(3)
Vb	266(240)	301(279)	394	433	672	70	7·36(4)	7·36(4)

[a] Minimum in parentheses; [b] by absorption; [c] by fluorescence, excited at the isosbestic point of the absorption spectrum.

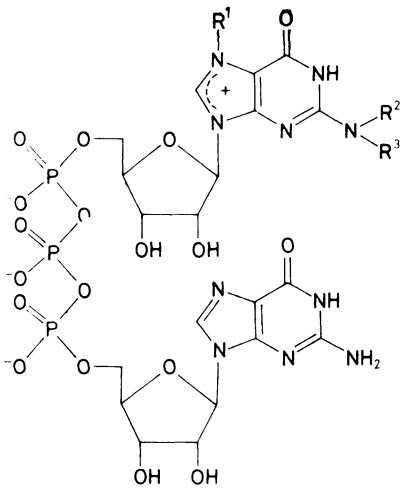
TABLE II

Thermodynamic parameters for intramolecular stacking of dinucleoside triphosphates (*Ia*—*Va*)

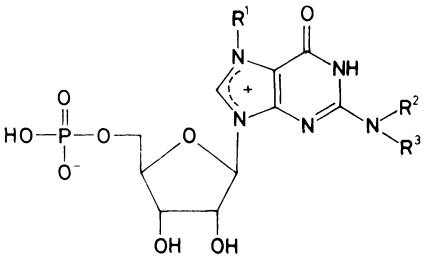
Compound	pH	K^a	ΔH^0/kJ mol l^{-1}	ΔS^0/J K^{-1} . mol^{-1}
Ia	5·06	0·43	−12·6(5)	−47(4)
	8·15	0·15	−14·2(17)	−65(5)
IIa	5·06	1·14	−9·8(3)	−31(1)
	8·86	0·74	−21·0(3)	−74(1)
IIIa	5·06	0·14	−20·5(3)	−86(2)
	8·86	no stacking observed		
IVa	5·06	2·05	−15·4(2)	−46(1)
	8·86	1·46	−20·3(3)	−66(1)
Va	5·06	3·19	−13·6(2)	−37(1)
	8·86	0·63	−29·2(5)	−93(4)

[a] K = [stacked]/[unstacked] at 298·2 K.

in a change in the wavelength of both absorption and fluorescence maximum and a marked decrease in the fluorescence intensity. The pK_a values based on these spectral changes are listed in the same table. With each compound, the acidity constant obtained fluorometrically is within the limits of experimental errors equal



a



b

Ia, *Ib*, $R^1 = CH_3$; $R^2 = R^3 = H$
IIa, *IIb*, $R^1 = CH_2CH_3$; $R^2 = R^3 = H$
IIIa, *IIIb*, $R^1 = CH_2C_6H_5$; $R^2 = R^3 = H$
IVa, *IVb*, $R^1 = R^2 = CH_3$; $R^3 = H$
Va, *Vb*, $R^1 = R^2 = R^3 = CH_3$

to that obtained spectrophotometrically. In other words, the fluorometrically determined values also refer to acidity of the ground state, as expected on the basis of relatively small quantum yield and short life-time of the excited state[2,4].

Table II summarizes the thermodynamic data obtained fluorometrically for intramolecular base-stacking of dinucleoside triphosphates, *Ia*–*Va*. The values of the equilibrium constant, K, are based on the assumption that the fluorescence intensity of the unstacked form equals to that of the corresponding nucleoside monophosphate, while the stacked form is not fluorescent, as discussed previously by Nishimura et al.[4]. The enthalpies and entropies of interaction were calculated from the equilibrium constants at different temperatures via Van't Hoff equation, assuming that ΔH^0 is independent of temperature. All the measurements were carried out at concentrations 5 to 8 . 10^{-5} mol l^{-1} where intermolecular association may be neglected. For comparison, the association constants of 7-methylguanosine and its 5'-monophosphate with purine bases have been reported to be of the order of 10 mol .

. l^{-1}. Accordingly, no marked stacking at concentrations less than 10^{-3} mol l^{-1} is expected to take place[5]. As seen from Table II, intramolecular stacking of dinucleoside triphosphates is a clearly exothermic process, but the favourable enthalpy change is largely compensated by an unfavourable interaction entropy. The cationic (N1 protonated) 7-alkylguanine residue stacks more efficiently than the zwitterionic (N1 deprotonated) one, analogously to intermolecular stacking of 7-methylguanosine or its 5'-monophosphate with purine bases[5]. It is noteworthy, however, that the enthalpy of interaction becomes more negative on deprotonation of N1, but the entropy of interaction undergoes a parallel change that overcompensates the enthalpy gain. It is also seen from Table II that methylation of the 7-alkyl guanine residue at N2 considrably enhances stacking, which may be accounted for by increased polarizability of the π-electron system[6]. Analogously, replacing the 7-methyl substituent with an ethyl group also strengthens intramolecular stacking. By contrast, substitution of N7 with a benzyl group results in a marked destacking compared to methyl and ethyl derivatives. One may tentatively assume that the phenyl ring, which is out of plane of the guanine ring, forms a steric hindrance to vertical association of the base residues.

The data in Table I reveal that the pK_a value of the 7-alkylguanine moiety is about 0·1 unit higher with dinucleoside triphosphates than with the corresponding nucleoside monophosphates. Possibly, the acidity difference is slightly larger with those compounds that exhibit strong intramolecular stacking, but the differences are far too small to allow any firm conclusions to be drawn.

EXPERIMENTAL

The absorption spectra were recorded on a Zeiss Specord M-40 spectrophotometer. The fluorescence spectra were measured by using a set-up in which the sample was excited frontally[7]. The cell housing block was thermostated within an accuracy of 1 K, and the temperature was measured directly from the cell with an thermoelement. The pH was adjusted with a phosphate buffer (0·05 mol l^{-1}) and checked with a pH-meter calibrated with commercial standard buffer solutions. The ionic strength was adjusted to 0·15 mol l^{-1} with NaCl.

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