INTERACTION OF THE tRNA-DERIVED UNUSUAL NUCLEOSIDE, t^6A, WITH Mn(II) AND Mg(II)

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1. Introduction

We have been interested for some time in the role certain unusual anticodon-adjacent nucleosides might play in the structure and functions of transfer RNA. One of these, *N*-[9-(β-D-ribofuranosyl) purin-6-ylcarbamoyl]threonine, t^6A ([1,2], fig.1) occurs at the 3′-end of the anticodon in most tRNAs which read codons beginning with adenosine [3]. We have shown in [4] that t^6A is required for optimal interaction of *Escherichia coli* Ile-tRNA^{Ile} with poly(AUC) on ribosomes. t^6A-deficient tRNA bound less extensively as a function of Mg(II), suggesting that the modified nucleoside could be serving as a metal ligand. We have also presented [5] qualitative evidence for the interaction of Mn(II) with t^6A by ^{13}C NMR. The paramagnetic Mn(II) ion was shown to bind primarily to the carboxyl group, with other possible coordination sites being N_7 and N_1 of the adenine ring and the ureido carbonyl oxygen of the sidechain. Though Mn(II) is considered in most instances a good substitute for Mg(II) in terms of biological activity and ionic size, it was thought important to investigate the interaction of Mg(II) with t^6A directly by other techniques. Here we report a study on the interaction of Mg(II) with t^6A using potentiometry. We have also studied the Mn(II) interaction with this ligand for comparison.

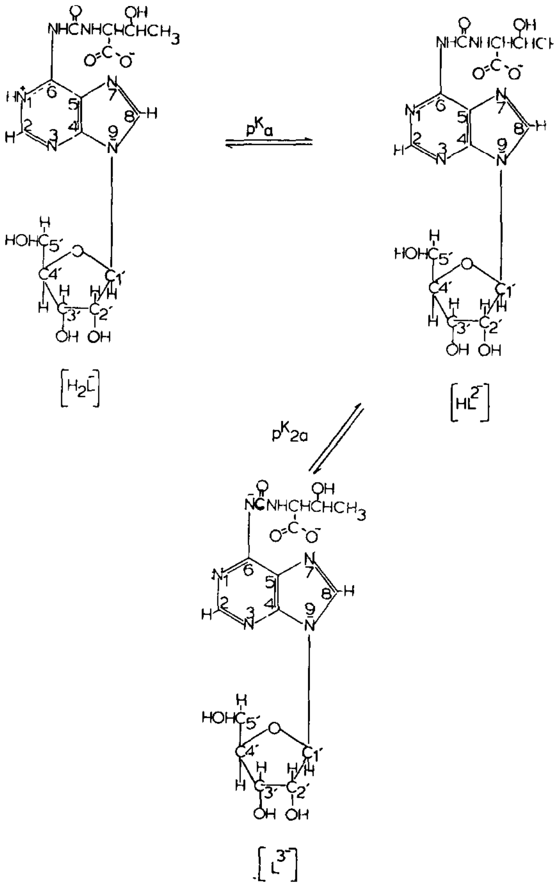

Fig.1. Schematic representation of the dissociations form t^6A. At pH 4, there is a partial positive charge, δ + at N_1 (although N_3 and N_7 are less basic than N_1, there is also undoubtedly some protonation at these sites at this pH) so that t^6A does have a net negative charge due to the carboxyl ionization. Therefore, t^6A at pH 4 is designated H_2L^-.

2. Materials and methods

The preparation of t^6A and m^6t^6A has been described [6a,b]. For every titration fresh solid ligand was weighed out, in order to avoid possible hydrolysis of the stock solution. Analytical grade metal salts were obtained from Mallinckrodt and were standardized volumetrically by titration with Na_2EDTA in the presence of a suitable indicator as outlined [7].

Potentiometric titration of the ligand was carried out with a standard NaOH solution in the absence and presence of the metal ion being investigated. The ionic strength was maintained constant by using 0.10 M KNO_3 as the supporting electrolyte and relatively low concentrations of ligand and metal ion. A stream of nitrogen was passed through the titration cell to exclude adverse effect of atmospheric CO_2. All titrations were carried out at 30 ± 0.1°C.

A Sargent-Welch pH meter with combination electrode was used to determine the pH. The electrode system was calibrated by direct titration of acetic acid and the observed pH meter reading was compared to the actual H^+ concentration determined from the pK_a value of acetic acid at 30°C as tabulated [8].

The regions of pH <3.5 and >10.5 were calibrated by measurements in HCl and NaOH solutions, respectively.

2.1. *Calculations – pK values of t^6A*

The acid dissociation constants for the two dissociable protons of t^6A (H_2L^-; refer to fig.1 for structures) are related to the usual equilibrium expressions:

$$H_2L^- \overset{K_a}{\rightleftharpoons} HL^{2-} + H^+ \tag{1}$$

$$HL^{2-} \overset{K_{2a}}{\rightleftharpoons} L^{3-} + H^+ \tag{2}$$

and the constants K_a and K_{2a} were calculated by the following equations:

$$K_a = \frac{[H^+]aT_L + [H^+]-[OH^-]}{T_L - aT_L + [H^+]-[OH^-]} \tag{3}$$

$$K_{2a} = \frac{[H^+](a-1)T_L + [H^+]-[OH^-]}{T_L-(a-1)T_L + [H^+]-[OH^-]} \tag{4}$$

Where T_L and a represent the total concentration of ligand species and the no. mol base added/mol t^6A, respectively.

2.2. *Binding constants*

In order to determine the stability constants of 1:1 metal–t^6A complexes, the following equations are used:

$$M^{2+} + HL^{2-} \rightleftharpoons MHL \tag{5}$$

$$M^{2+} + L^{3-} \rightleftharpoons ML^- \tag{6}$$

Related equilibria may be described as:

$$M^{2+} + H_2L^- \rightleftharpoons MHL + H^+ \tag{7}$$

$$MHL \rightleftharpoons ML^- + H^+ \tag{8}$$

The following expressions may be written, between $a = 0$ and $a = 1$, assuming that a protonated 1:1 complex species (MHL) is formed.

If T_L represents the total concentration of various ligand species and T_M that of all metal species then:

$$T_L = [H_2L^-] + [HL^{2-}] + [MHL] \tag{9}$$

$$T_M = [M^{2+}] + [MHL] \tag{10}$$

The total amount of titratable hydrogens:

$$[H^+] = [HL^{2-}] + [MHL]-aT_L \tag{11}$$

In the buffer region between $a = 1$ and $a = 2$, a simple dissociation of the [MHL] species was assumed and the following equations are set up:

$$T_L = [HL^{2-}] + [L^{3-}] + [ML^-] \tag{12}$$

$$T_M = [M^{2+}] + [ML^-] \tag{13}$$

the total titratable hydrogens are:

$$[H^+] = [L^{3-}] + [ML^-]-T_L + [OH^-] \tag{14}$$

By solving suitable material balance equations the final expression for K^M_{MHL} and K_1, the protonated and normal 1:1 complexes, respectively, can be obtained as:

$$K^{M}_{MHL} = \frac{T_M - [M^{2+}]}{[M^{2+}]\left\{(1-a)\,T_L - [H^+] + [OH^-] \Big/ \dfrac{[H^+]^2}{K_a K_{2a}} + \dfrac{[H^+]}{K_{2a}}\right\}}$$

$$K_1 = \frac{T_M - [M^{2+}]}{[M^{2+}]\left\{(1-a)\,T_L - [H^+] + [OH^-] \Big/ \dfrac{[H^+]}{K_{2a}}\right\}}$$

3. Results and discussion

3.1. *Determination of pK values of t^6A*

The potentiometric titration curve of $t^6A(H_2L^-)$ in fig.2 shows a steep inflection at $a = 1$ followed by a buffer region at higher pH, indicating a stepwise dissociation of protons corresponding to the separate neutralized reactions. The dissociation constants calculated from eq. (3) and (4), are listed in table 1. Previous investigators [1] have also shown that there exist two dissociations, one in acidic and other in alkaline medium, based on ultraviolet absorption spectra.

In fig.2 is also given the titration curve of t^6A with Mg(II) in a 1:1 ratio of ligand to metal concentration. A similar titration curve was obtained for Mn(II) with t^6A. The curves show a steep inflection at $a = 1$ followed by a buffer region at high pH. Accordingly, it was assumed, that a monoprotonated 1:1 metal chelate species is formed in the buffer region ($a = 0$ and $a = 1$). In the buffer region between $a = 1$ and $a = 2$, dissociation of the monoprotonated species has been assumed. The constants, thus calculated, are listed in table 1. The first dissociation of t^6A, $pK_a = 3.35$, probably involves the removal of a proton from the basic center at N_1 of the pyrimidine ring. This is in accord with data on related systems [9]. The second dissociation may involve the N_6 proton, with a $pK_{2a} = 8.55$. Our pK_a value of 3.35 is comparable to the pK_a of 3.89 reported for adenine [9]. The pK_{2a} value of 8.55 is in close agreement with the pK_{2a} value of 8.48 reported for the dissociation of N_1–H of inosine at 35°C and 0.10 ionic strength [10]. The close agreement with the reported values on similar systems further supports the suggested dissociations of t^6A.

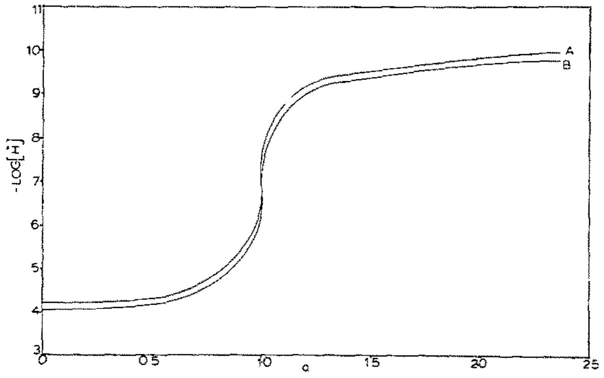

Fig.2. Potentiometric titration of t^6A with Mg(II) at a 1:1 ratio of ligand to metal ion; $t = 30°C$; ionic strength = 0.10 M (KNO_3); A = free t^6A; B = Mg(II) + t^6A; and a = no. mol base added/mol t^6A.

Table 1
Equilibrium constants for the interaction of Mn(II) and Mg(II) with t^6A: $pK_a = 3.35 \pm 0.01$; $pK_{2a} = 8.55 \pm 0.02$

Metal ion	Log K^{Ma}_{MHL}	Log K_1^a
Mn(II)	3.9	6.0
Mg(II)	3.8	5.5

[a] The constants are accurate to ± 0.1 log K unit

$t = 30°C$, ionic strength = 0.10 M (KNO_3)

Finally, it is of interest here to compare the pK values of m^6t^6A [11] with those of t^6A. The first dissociation constant of m^6t^6A is the same as that of t^6A. But the second dissociation from m^6t^6A is much higher, 10.15, as compared with 8.55, and may be from the hydroxyl group. The only difference between m^6t^6A and t^5A is that the former has a methyl group instead of a proton at N_6. These results confirm our assignment of the second ionization of t^6A.

It may be seen from table 1 that Mn(II) forms more stable complexes than Mg(II) with t^6A, a trend which is expected based on the reactive tendencies between the two metal ions. Though there is a slight difference in the order of stability for the protonated 1:1 complexes, there is a considerable enhancement of stability in the case of Mn(II) as compared to Mg(II) for the normal 1:1 chelates. This shows that the binding capacity of Mn(II) differs from that of Mg(II) at a pH which is biologically important. The ligand sites, however, are considered to be equivalent. Of the various possible sites, base, ribose and side chain, the ribose appears to be the weakest donor group. The metal may bind, therefore, to the more probable sites, viz., the carboxylate group of the side chain and N_7 of the imidazole ring or N_1 of the pyrimidine ring. Support for the former comes from our studies on the Mn(II) interaction with t^6A by ^{13}C NMR [5]. We have shown that the line broadening effect upon progressive addition of Mn(II) to t^6A at pH 7.4, was most dramatic at the carboxyl carbon signal followed by C_5, indicating preferential binding to the carboxyl group and N_7.

The results reported here and in our NMR study definitely show that cation binding to t^6A occurs. The stability constants for the neutral Mg(II) and Mn(II) complexes (MHL) are significant. This metal liganding may portray a facet of the functional role of t^6A in tRNA. Our findings are in contrast to those reported [12] on the dinucleoside monophosphate Upt^6A. These authors found no evidence of cation binding to the dimer or the 5′-monophosphate, pt^6A, using CD or ultraviolet absorption. They stated that the ΔA upon N_1 protonation (presumably analogous to metal ion binding) was less than upon protonation of the carboxyl group. This seems somewhat novel in light of the fact that N_1 is intimate to the chromophore whereas the carboxyl is out on the side chain. The reasons for the discrepancies remain to be resolved.

Acknowledgements

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