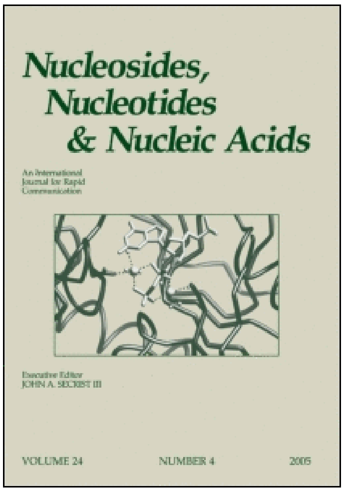

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PROPERTIES OF SOME DIURIDINE PHOSPHATE ANALOGUES

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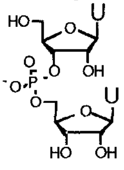
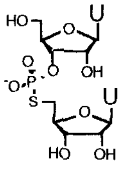
ABSTRACT: The hydrolytic stability of oligoribonucleotides containing 2′- amino nucleophile is due to poor leaving characteristic of 5′-nucleoside, replacement of 5′-leaving group by thio or amino results in considerable instability towards hydrolysis.

INTRODUCTION

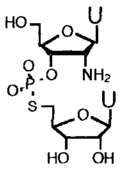
Polyribonucleotides and oligoribonucleotides containing a 2′-amino group as opposed to a 2′-hydroxyl group, are stable to alkaline, RNaseA and hammerhead ribozyme-induced hydrolyses.[1-2] This lack of reactivity is rather curious since the 2′-amino group has a pKa of 6.2 in 2′-aminouridine[3] and in amUpT[4,5], therefore at pH 8 it is not protonated and should be a good nucleophile for transphopshorylation reaction. To determine whether cleavage could be obtained with the 2′-amino group by variation of the leaving group we have synthesised the following UpU analogues.



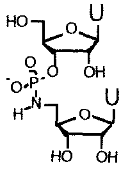
UpU



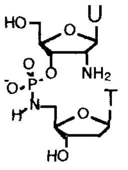
UpsU



amUpsU



UpnU



amUpnT

RESULTS AND DISCUSSION

Hydrolytic Stability: The analogues synthesised have a 5′-thio leaving group in conjunction with a 2′-hydroxy and 2′-amino nucleophile in UpsU and amUpsU, respectively, and a 2′-hydroxy and 2′-amino nucleophile in conjunction with a 5′-amino leaving group in UpnU and amUpnT respectively.[6]

In contrast to the hydrolytic stability of UpU and amUpT, UpsU, amUpsU, UpnU and amUpnT were readily hydrolysed. UpsU is easily hydrolysed, the reaction rate increases linearly with pH due to the increased concentration of 2′-oxy anion species at higher pH.[6] Hydrolysis of amUpsU follows two distinct pathways, with the final hydrolysis products being dependent upon the pH of the reaction mixture. It is pH independent above pH 6.4, because the 2′-amine remains unprotonated above its pKa. As the pH falls because of protonation of the amino group the hydrolysis rate is reduced in direct proportion to pH, Table-I.

Thus, the hydrolysis of UpsU and amUpsU compared to UpU and amUpT, is clearly more facile due to the differences in pKa between the 5'-thio (pKa = 11) and the 5'-oxo (pKa = 16) leaving group and much lower P-S bond strength, approx. 50 kcal mol^{-1}, compared to the P-O and P-N bond 91 and 70 kcal mol^{-1} respectively.

For the hydrolysis of UpnU and amUpnT cleavage of the P-N bond appears to be the rate limiting step. At lower pH this nitrogen is readily protonated, thus rendering the P-N bond extremely labile. At higher pH, above 6.2, there is lower concentration of protonated species and the hydrolysis rate for UpnU levels off, which reflects the poor leaving ability of RNH^-. The dinucleotide amUpnT is more readily hydrolysed than UpnU and it is not until above pH 10 that amUpnT exhibits greater hydrolytic stability than UpnU. Thus, the 2'-oxo anion in UpnU more readily displaces the 5'-amine than does the 2'-amino group in amUpnT, as expected from the difference in P-O and P-N bond strengths.

Metal ion enhancement: Irrespective of the metal ions (Mg^{2+}, Zn^{2+} and Cd^{2+}) used the hydrolysis rate increases. The approximately 2-, 13- and 8 fold rate enhancement for UpsU and 2-, 10- and 12 fold for UpnU

TABLE-I:Rate of dinucleotide hydrolysis K_{obs} (10^{-6} s^{-1}) with pH

pH	5.0	5.6	6.0	7.0	7.5	8.0	9.0	10.0	11.5
UpU	-	-	-	-	-	-	-	-	14
UpnU	1802	913	292	242	140	138	101	98	109
UpsU	*b*	*b*	5	51	97	504	3200	*a*	*a*
amUpsU	14	-	40	85	89	93	116	128	138
amUpnT	*a*	*a*	*a*	11685	2404	1562	587	54	17

a: Above pH 9 for UpsU and below pH 7 for amUpnT hydrolysis was too fast ; b: below pH 6 for UpsU hydrolysis was too slow to measure.

is observed as compared to no metal ion present. The observed rate enhancements in UpsU hydrolysis do not correlate with what would be expected from increased general-base catalysis, since the pKa values are 11.4, 8.8 and 9.0 for the aquo/hydroxy complexes of Mg^{2+}, Zn^{2+} and Cd^{2+} respectively.

The results for amUpsU hydrolysis with Cd^{2+} is consistent with the concept that Cd^{2+} can stabilise the developing negative charge on the 5'-thio- leaving group. However, it is not clear why this enhancement does not also manifest itself in UpsU hydrolysis.[6]

RNase A Stability: The three cleavable dinucleotides UpU, UpsU and UpnU has only marginal differences in their K_M values, which suggests that association of the substrate with the enzyme in the ground state is approximately the same for all of them. A 35-fold higher k_{cat} for UpnU compared to UpU and UpsU, is due to easy protonation of the 5′-amino-nucleoside in the active site of the enzyme by the general acid His-119.

Inspite of the lower P-S bond strength UpsU is cleaved by RNase A at a similar rate as UpU because the 5′-thio-nucleoside with pKa *ca.* 10 is much more difficult to protonate than a 5′-oxo-nucleoside with pKa *ca.*15. It is interesting to note that amUpsU is not a substrate for RNaseA.

CONCLUSION

The dinucleotides amUpsU and amUpnT are readily hydrolysed in contrast to amUpT, indicating the importance of the leaving group when the 2'-amino group is the nucleophile. The metal ion effect of Cd^{2+} on the hydrolysis of amUpsU is a clear example for metal ion interaction with the leaving group. UpnU demonstrated a marked rate enhancement, relative to UpU and UpsU, when cleaved by RNase A consistent with the postulated protonation of the 5'-amino leaving group in the hydrolysis of UpnU and amUpnT.

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