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SHORT COMMUNICATION

ACID-CATALYSED DEPURINATION OF DI-, TRI- AND POLYDEOXYRIBONUCLEOTIDES: EFFECT OF MOLECULAR ENVIRONMENT ON THE CLEAVAGE OF ADENINE RESIDUE

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First-order rate constants for the acid-catalysed cleavage of adenine base from 2′-deoxyadenosine, its 3′- and 5′-monophosphates, various dinucleoside monophosphates, trinucleoside diphosphates and hexameric 2′-deoxyadenylic acid were measured by the method of initial velocity. The results obtained were compared with the effect that heteroassociation with caffeine has on the pre-equilibrium protonation and rate-limiting heterolysis of 2′-deoxyadenosine and its isosteric analogue, 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole. The kinetics of the depurination of poly(dA–T), poly(dA) and a mixture of poly(dA) and poly(T) were determined, and the rate variations observed are discussed on the basis of the known structures of these polymers.

Cleavage of the purine bases adenine (Ade) and guanine (Gua) from DNA by acid-catalysed hydrolysis of the *N*-glycosidic bond of purine 2′-deoxyribonucleoside residues (**1a** and **b**) is one of the central chemical reactions of nucleic acids. It is encountered as a harmful side-reaction during chemical synthesis of oligodeoxyribonucleotides; removal of the sugar moiety protecting groups under acidic conditions is accompanied by partial depurination of the oligomer.[1] The same reaction has also been suggested to be a potential source of spontaneous mutagenesis;[2] an apurinic site in DNA may result in an error in replication. Mechanistically the reaction involves a rapid initial protonation of the base moiety and rate-limiting unimolecular rupture of the *N*-glycosidic bond (Scheme 1), as originally shown by Zoltewicz *et al.*[3] and subsequently supported by several lines of evidence.[4] The kinetic results available, however, refer only to monomeric nucleosides, and no attempts have been made to determine the depurination rate of 2′-deoxyribonucleosides incorporated in oligonucleotides. This paper is aimed at partly filling this gap. Rate constants for the cleavage of adenine base from 2′-deoxyadenosine (dA, **1a**), its 3′- and 5′-monophosphates (3′-dAMP, **2a**; 5′-dAMP, **2b**), various dinucleoside monophosphates (**3a–e**), trinucleoside diphosphates (**4a** and **b**) and hexameric 2′-deoxyadenylic acid (**5a**) were determined by the method of initial velocity. Moreover, depurination of a copolymer, poly(dA–T) (**5d**), and a homopolymer, poly(dA) (**5b**), in the absence and presence of poly(T) (**5c**), was studied. The results obtained are compared with the effect that heteroassociation with caffeine (**6**) has on the pre-equilibrium protonation and rate-limiting cleavage of dA and its isosteric analogue, 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**7**).

As can be seen from Table 1, dinucleoside monophosphates derived from either 3′-dAMP or 5′-dAMP (**3a–e**) are less susceptible to acid-catalysed

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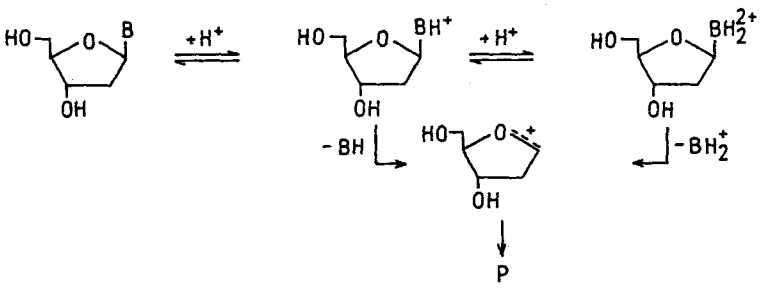
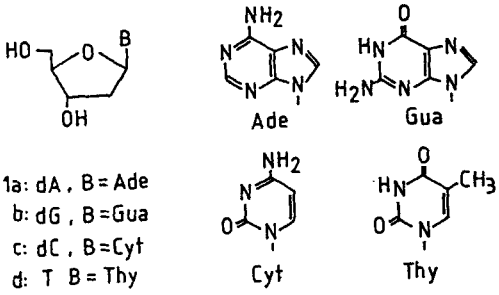
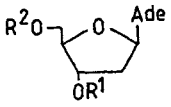


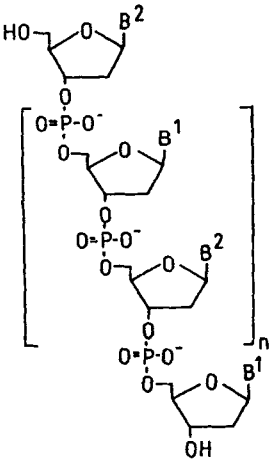
Scheme 1



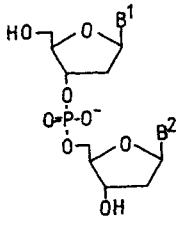
1a: dA, B = Ade
b: dG, B = Gua
c: dC, B = Cyt
d: T B = Thy



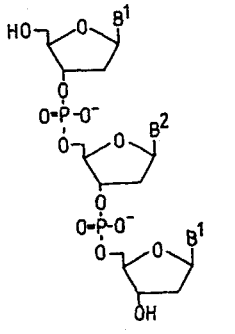
2a: 3'-dAMP, $R^1 = PO(OH)_2$, $R^2 = H$
b: 5'-dAMP, $R^1 = H$, $R^2 = PO(OH)_2$



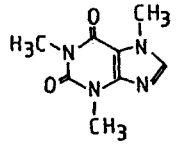
5a: $B^1 = B^2$ = Ade, n = 2
b: $B^1 = B^2$ = Ade, n ~ 100
c: $B^1 = B^2$ = Thy, n ~ 100
d: B^1 = Ade, B^2 = Thy, n ~ 100



3a: B^1 = Ade, B^2 = Thy
b: B^1 = Thy, B^2 = Ade
c: B^1 = Ade, B^2 = Cyt
d: B^1 = Cyt, B^2 = Ade
e: B^1 = Ade, B^2 = Ade



4a: B^1 = Thy, B^2 = Ade
b: $B^1 = B^2$ = Ade



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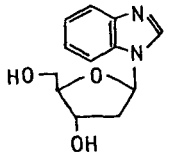

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depurination than dA. The relative depurination rates compared with dA at pH 2 and 303·2 K are dApT (**3a**) 0·30, TpdA (**3b**) 0·35, dApdC (**3c**) 0·21, dCpdA (**3d**) 0·35 and dApdA (**3e**) 0·26. On going to trinucleoside diphosphates the depurination is further decelerated, the relative rates being TpdApT (**4a**) 0·10 and dApdApdA (**4b**) 0·19. A considerable part of these rate

retardations may be attributed to phosphorylation of a sugar hydroxyl function; the depurination rate of both 3′-dAMP and 5′-dAMP is about half that of dA. The latter retardation results from destabilization of the oxocarbenium ion intermediate rather than from decreased basicity of the base moiety, since the pK_a value of 3′-AMP is known to be equal to that of adenosine (3·6 at 298·2 K, $I = 0{\cdot}1$ mol dm^{-3}), and 5′-AMP is even slightly more basic ($pK_a = 3{\cdot}8$ at 298·2 K, $I = 0.1$ mol dm^{-3}).[5,6]

The origin of the higher hydrolytic stability of dinucleoside monophosphates (**3a–e**) and trinucleoside

Table 1. First-order rate constants[a] for the acid-catalysed depurination of 2′-deoxyadenosine, its monophosphates and various di-, tri- and oligonucleotides

Compound	T/K	$-\text{Log}([H^+]/\text{mol dm}^{-3})$	$k_{obs}/10^{-6}$ s^{-1}
dAdo (**1a**)	303·2	2·00[b]	22·3 ± 0·6[d]
	303·2	4·55[c]	0·136 ± 0·003
	363·2	4·71[c]	45·3 ± 0·6
3′-dAMP (**2a**)	303·2	2·00	13·4 ± 0·3
	303·2	4·55	0·052 ± 0·003
5′-dAMP (**2b**)	303·2	2·00	12·5 ± 0·4
	303·2	4·55	0·059 ± 0·003
dApT (**3a**)	303·2	2·00	6·7 ± 0·3
	303·2	4·55	0·036 ± 0·003
	363·2	4·71	14·2 ± 0·3
TpdA (**3b**)	303·2	2·00	7·7 ± 0·3
	303·2	4·55	0·035 ± 0·002
	363·2	4·71	16·8 ± 0·3
dApdC (**3c**)	303·2	2·00	4·6 ± 0·5
	363·2	4·71	17·3 ± 0·1
dCpdA (**3d**)	303·2	2·00	7·8 ± 1·2
	363·2	4·71	21·9 ± 0·6
dApdA (**3e**)	303·2	2·00	5·9 ± 0·8
TpdApT (**4a**)	303·2	2·00	2·3 ± 0·1
dApdApdA (**4b**)	303·2	2·00	5·3 ± 0·1
dAp(dAp)$_4$dA (**5a**)	303·2	2·00	4·3 ± 0·1
Poly(dA) (**5b**)	303·2	2·00	0·10 ± 0·05
Poly(dA) + poly(T) (**5b** + **5c**)	303·2	2·00	25 ± 3
Poly(dA–T) (**5d**)	303·2	2·00	3·2 ± 0·4

[a] Obtained by the method of initial velocity.
[b] Adjusted with hydrogen chloride, $I = 0{\cdot}1$ mol dm^{-3} with sodium chloride.
[c] Adjusted with an acetic acid–sodium acetate buffer, $I = 0{\cdot}1$ mol dm^{-3} with sodium chloride.
[d] Standard deviation of mean of 12 samples.

Table 2. First-order rate constants for the acid-catalysed depurination of 2′-deoxyadenosine (**1a**) and 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**7**), and the pK_a values of N-1-protonated 2′-deoxyadenosine at various concentrations of caffeine (**6**)

[**6**]/mol dm^{-3}	k_{obs}(**1a**)/10^{-4} s^{-1} [a]	k_{obs}(**1a**)/10^{-6} s^{-1} [b]	k_{obs}(**7**)/10^{-6} s^{-1} [c]	pK_a(**1a**)[d]
—	2·70 ± 0·03[e]	1·25 ± 0·07	0·64 ± 0·04	3·66
0·0125	2·54 ± 0·02	1·21 ± 0·07	0·87 ± 0·05	3·49
0·025	2·56 ± 0·02	1·14 ± 0·07	0·94 ± 0·05	3·41
0·050	2·35 ± 0·03	1·05 ± 0·06	0·87 ± 0·07	3·30
0·075	2·44 ± 0·04	0·97 ± 0·06	1·07 ± 0·05	
0·100	2·31 ± 0·01	0·92 ± 0·06	—	3·17

[a] In hydrochloric acid (0·1 mol dm^{-3}) at 303·2 K.
[b] In acetic acid–sodium acetate (0·5 and 0·1 mol dm^{-3}, respectively) buffer at 313·2 K.
[c] In hydrochloric acid (0·1 mol dm^{-3}) at 313·2 K.
[d] At 298·2 K; $I = 0{\cdot}1$ mol dm^{-3} with sodium chloride.
[e] Standard deviation of mean of 12 samples.

diphosphates (**4a** and **b**) compared with 3′-dAMP and 5′-dAMP is of considerable interest. Factors that may affect the depurination rate include intramolecular base stacking and/or intramolecular electrostatic interactions. With dApT (**3a**), TpdA (**3b**) and TpdApT (**4a**), the thymine base (Thy) remains uncharged under the experimental conditions employed,[7] and hence the electrostatic interactions between the base moieties may be ignored. The population of the stacked form of dinucleoside monophosphates has been estimated to vary from 20 to 40% at 293 K, except for uridylyl-(3′,5′)uridine, the stacked population of which is less than 10%.[8] To elucidate the effect that base stacking may have on the pre-equilibrium protonation and rate-limiting departure of the adenine ring, the pK_a values of dA and the first-order rate constants of its depurination were determined at various concentrations of caffeine (**6**). The results obtained are summarized in Table 2.

Caffeine is known to stack efficiently with adenine derivatives, the association constant with adenosine, for example, being 39 $dm^3 mol^{-1}$ at 298·2 K.[9] Accordingly, dA may be expected to be largely stacked at a caffeine concentration of 0·1 mol dm^{-3}. As can be seen from Table 2, the pK_a, value of dA, which refers to N-1 protonation,[7] is markedly decreased with increasing caffeine concentration, suggesting that stacking of the adenine ring with caffeine reduces its basicity, and hence retards depurination. The observed first-order rate constant is also decreased with increasing caffeine concentration, but considerably less than the equilibrium constant of the initial protonation. In other words, base-stacking interactions appear to retard the initial protonation and facilitate the departure of the protonated base moiety, the former influence being more marked. To verify this conclusion, the effect of caffeine concentration on the hydrolysis of 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole (**7**) was studied. This compound reacts in principle by the same mechanism as dA.[10] However, it contains only one potential site of protonation, and hence the hydrolysis rate becomes independent of pH at $pH < pK_a$ ($pK_a = 3·9$ at 363·2 K).[10] Under these conditions, the observed first-order rate constant is equal to the rate constant for the unimolecular heterolysis of the protonated substrate, which may be regarded as a model of the rate-limiting stage of dA depurination. The data in Table 2 show that the presence of caffeine indeed accelerates the heterolysis of the conjugate acid of **7** by about 50%. Accordingly, the conclusion that base stacking retards the protonation of dA, and that this rate retardation is partly compensated for by acceleration of the rate-limiting heterolysis, appears to be valid.

Since intermolecular stacking of dA with caffeine reduces its depurination rate by only about 20%, it appears evident that intramolecular base stacking may have only a slight effect on the hydrolytic stability of dApT (**3a**), TpdA (**3b**) and TpdApT (**4a**). The stacking population of the latter compounds is undoubtedly less than 40%,[8] and hence the rate retardation may be expected to be of the order of 10%. Consistent with this view, the relative depurination rates of **3a** and **3b** compared with dA are not markedly increased with temperature, although the stacked population at elevated temperatures (350 K) is only about half of that at room temperature.[8] The relative rates observed at 303·2 and 363·2 K are 0·26 and 0·31 with **3a** and 0·26 and 0·37 with **3b**.

With dApdC (**3c**), dCpdA (**3d**) and dApdA (**3e**), both of the base residues are positively charged at pH 2; adenine bears a proton at N-1 and cytosine at N-3. The log β_2 value for the formation of diprotonated ApA, the ribo analogue of **5c**, was observed to be 6·8 ± 0·1 at 298·2 K ($I = 0·1$ mol dm^{-3}). The pK_a value of the diprotonated form is thus 3·2, assuming that the pK_a value of the monoprotonated species is equal to that of adenosine (3·6). Accordingly, protonation of one of the base moieties reduces the basicity of the remaining adenine residue by 0·4 unit. This effect is large enough to account for the increased hydrolytic stability of **3c–e** compared with 3′- and 5′-dAMP. Trimeric (**4b**) and hexameric 2′-deoxyadenylic acids (**5a**) are depurinated only slightly more slowly than the corresponding dimer (**3e**).

The depurination rate of copolymer poly(dA–T) (**5d**) does not differ markedly from that of the corresponding trimer, TpdApT (**4a**). It has been shown[11] that this polymer has a single-stranded structure under acidic conditions, and hence the result is expected. In contrast, poly(dA) (**5b**) adopts a double stranded helical structure at pH 2.[12,13] It is interesting that the depurination rate of this double-stranded polymer is only 3% of that of the single-stranded copolymer **5d**. However, the data available do not allow one to decide whether the high hydrolytic stability is a general feature of double-stranded helices. Surprisingly, poly(dA) is depurinated in the presence of one equivalent of poly(T) (**5c**) over 200 times as fast as in the absence of **5c**. According to hypochromicity measurements, poly(dA) is unable to complex with polyuridylic acid at $pH < 3$.[13] This finding strongly suggests that poly(dA) would also be unable to complex with poly(T) at pH 2. However, the exceptionally high depurination rate cannot be understood without assuming some kind of interaction between poly(dA) and poly(T).

In summary, adjacent nucleoside residues have only a moderate effect on the rate of acid-catalysed depurination of 2′-deoxyadenosine residues of small oligodeoxyribonucleotides. The effect of electrostatic interactions between adjacent bases appears to be more important than base stacking. At the polymeric level inter-strand interactions may either accelerate or decelerate the depurination by one order of magnitude.

EXPERIMENTAL

Materials. The trinucleoside diphosphates (**4a** and **b**) and hexameric oligo(dA) (**5a**) were prepared by the manual phosphite–triester method,[1] using 3·0 μmol of the appropriately derivatized CPG support (Sigma, type 1), 0·1 mol dm^{-3} 5′-*O*-dimethoxytrityl-2′-deoxynucleoside 3′-(2-cyanoethyl)diisopropylamidophosphite and 0·4 mol dm^{-3} tetrazole on a Cruachem PS 100 DNA synthesizer. After deprotection, the oligonucleotides were purified by ion-exchange high-performance liquid chromatography (HPLC) on a Synchropak AX-300 column [250 × 4·6 mm i.d.; flow-rate 1·0 cm^3 min^{-1}; eluent A, KH_2PO_4 (0·03 mol dm^{-3}) in 50% aqueous formamide (pH 5·6); eluent B, KH_2PO_4 (0·03 mol dm^{-3}) and $(NH_4)_2SO_4$ (0·6 mol dm^{-3}) in 50% aqueous formamide (pH 5·6)], desalted (Alltech Maxi-Clean C_{18} cartridge) and evaporated to dryness. All the other nucleosides, nucleotides and polynucleotides were commercial products from Sigma. They were used as received, after checking their purity by HPLC. The preparation of 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)benzimidazole has been described previously.[10] Caffeine was a product of Aldrich (99%).

Determination of pK_a values. The pK_a values were determined by the potentiostatic method described previously.[14]

Kinetic measurements. First-order rate constants of the depurination were obtained by the method of initial velocity. The progress of the reaction was followed by withdrawing aliquots from the reaction mixture as long as about 10% of the adenine residues were released. The composition of the samples was analysed by the HPLC technique described previously,[4] and the adenine content of each aliquot was compared to that observed after complete depurination of the starting material. The initial substrate concentration was about 5×10^{-4} mol dm^{-3}, expressed as the amount of adenine released in complete hydrolysis. Chromatographic separations were carried out on a Hypersil ODS column (250 × 4·6 mm i.d., 5 μm), using aqueous ammonium acetate (0·1 mol dm^{-3}) containing 5% (v/v) of acetonitrile as eluent.

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