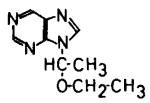
Effect of Base-stacking on the Alkaline Cleavage of 9-(1-Ethoxyethyl)purine *

HARRI LÖNNBERG and JYRKI YLIKOSKI

Department of Chemistry and Biochemistry, University of Turku, SF-20500 Turku, Finland

Nucleic acid bases,[2-6] nucleosides[3,7-11] and nucleotides[12,13] associate markedly in aqueous solution. The associates consist of several base molecules stacked vertically, *i.e.* perpendicular to the plane of the stacking species.[7,8,14,15] Dipole induced dipole interactions have been suggested to be the driving force for the association.[16] In addition, hydrophobic bonding may contribute.[2,5,6,8,11,17,18] This kind of stacking may be expected to affect the reactivity of the associated molecules, but practically no quantitative information about the subject exists. Nagy and co-workers[19] studied the effect of charge-transfer complexing agents on the acidic hydrolysis of adenosine, 2′-deoxyadenosine and 2′-deoxyguanosine, but observed only small influences, both rate-retarding and -enhancing, at the low solute concentrations employed (<0.1 mol dm^{-3}).

We have previously[20,21] suggested that the alkaline hydrolysis of an acyclic nucleoside analogue, 9-(1-ethoxyethyl)purine (*1*),



(*1*)

involves a rate-limiting attack of a hydroxide ion on C8 of the purine moiety, a rapid subsequent departure of the attacked carbon atom, a formate ion, and a rapid cyclization to 7,8-dihydro-8-methylpurine with concomitant loss of the ethoxy group. In the present communication we show that association with heteroaromatic cosolutes retards the decomposition of the starting material, *i.e.* the nucleophilic attack of the hydroxide ion on the purine ring.

Table 1 summarizes the influences that various neutral and anionic cosolutes exert on the rate constant for the hydrolysis of 9-(1-ethoxyethyl)purine in aqueous sodium hydroxide. Pyridine, pyrimidine and 1-(1-ethoxyethyl)cytosine have practically no effect. In contrast, alkylated benzimidazoles and derivatives of adenine retard the disappearance of the starting material significantly. Methylpyridines are also rate-retarding, but the influence is small. The equilibrium constants for the association of the cosolutes with free purine[22] are also included in Table 1. Comparison with the kinetic data clearly indicates that the rate-retarding cosolutes are those capable of forming stable associates with purine, while the weakly associating species exhibit practically no effect on the hydrolysis rate. For pyridines no information about the stacking behavior is available, but the observed cosolute influences are consistent with the finding[23] that alkyl substituents enforce the interactions between pyridines and aromatic molecules.

As seen from Table 1, the anionic cosolutes, *viz.* the monoanions of purine and adenine, are rate-retarding as well. Obviously the major part of the observed influences, however, result from common electrolyte effects. For comparison, sodium perchlorate and sodium acetate diminish the observed rate constant at the concentration of 0.2 mol dm^{-3} by 20 and 15 %, respectively. This is expected, since the distribution of the negative charge is increased on going from the reactants to the transition state.[24] However, part of the kinetic cosolute effects of the purine and adenine monoanions may be attributed to the stacking phenomenon.

* See Ref. 1.

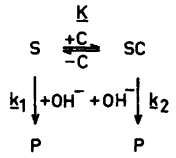
0302-4369/85 $2.50

Table 1: The effect of heteroaromatic consolutes, C, on the hydrolysis of 9-(1-ethoxyethyl)purine in aqueous sodium hydroxide at 343.2 K.[a]

C	[C]/mol dm^{-3}	$\frac{k\ (\text{obs.})}{k_1\ [OH^-]}$	$\frac{k_2}{k_1}$	K/dm^3 mol^{-1}	b
Pyridine	0.025	1.03[c]			
	0.050	0.97			
	0.075	0.97			
	0.100	0.95			
2,6-Dimethylpyridine	0.025	0.98			
	0.050	0.97			
	0.075	0.93			
	0.100	0.94			
2,3,6-Trimethyl-pyridine	0.025	0.97 (0.96)[d]	0.63	4.9	
	0.050	0.93 (0.93)			
	0.075	0.88 (0.90)			
	0.100	0.89 (0.88)			
Pyrimidine	0.025	0.98			0.5
	0.050	0.99			
	0.075	0.99			
	0.100	0.97			
1-(1-Ethoxyethyl)-cytosine	0.050	1.03			0.5[e]
	0.100	0.96			
1-Methylbenzimidazole	0.025	0.95 (0.93)	0.61	8.2	9.8
	0.050	0.87 (0.89)			
	0.075	0.85 (0.85)			
	0.100	0.83 (0.82)			
1,2-Dimethylbenz-imidazole	0.025	0.94 (0.94)	0.71	10.2	12.9
	0.050	0.90 (0.90)			
	0.075	0.88 (0.87)			
	0.100	0.85 (0.85)			
9-Methyladenine	0.025	0.94 (0.94)	0.65	7.9	11.7[e]
	0.050	0.91 (0.90)			
	0.075	0.86 (0.87)			
	0.100	0.85 (0.85)			
8,9-Dimethyladenine	0.025	0.94 (0.94)	0.79	15.4	17.7[e]
	0.050	0.91 (0.91)			
	0.075	0.89 (0.89)			
	0.100	0.87 (0.87)			
6-Dimethylamino-9-ethoxymethylpurine	0.025	0.92 (0.91)	0.68	16.4	19.3[e]
	0.050	0.84 (0.86)			
	0.075	0.82 (0.82)			
	0.100	0.81 (0.80)			
Sodium salt of purine	0.025	0.92			
	0.050	0.86			
	0.075	0.84			
	0.100	0.82			
	0.150	0.78			
	0.200	0.75			

Sodium salt of adenine	0.025	0.94
	0.050	0.89
	0.075	0.87
	0.100	0.83
	0.125	0.80
	0.150	0.78
	0.175	0.76
	0.200	0.68
Sodium perchlorate	0.200	0.80
Sodium acetate	0.200	0.85

[a] For k_1, k_2 and K see Scheme 1. k (obs.) denotes the observed pseudo first-order rate constant. [b] Equilibrium constant ($dm^3\ mol^{-1}$) for the association of C with free purine in aqueous solution at 298.2 K. [c] Means of duplicate measurements. [d] Calculated *via* eqn. (2) using the parameters listed. [e] From Ref. 22.



The cosolute effects described above can well be accounted for by the reaction pathway depicted in Scheme 1, where S and C stand for the substrate and cosolute, respectively. Under the pseudo first-order conditions, *i.e.* when [S] ≪ [OH^-] and [C], the rate-law obeyed may be expressed by eqn. (1). Here K is the equilibrium constant for the formation of the

$$\frac{d[P]}{dt} = \frac{k_1[OH^-] + k_2K[OH^-][C]}{1+K[C]}\,[S(tot.)] \qquad (1)$$

associate, SC, and k_1 and k_2 are the second-order rate constants for the decomposition of the free and associated substrate, respectively. The dependence of the observed pseudo first-order rate constant, k(obs.), on the cosolute concentration may thus be described by eqn. (2). The least-squares fitting with the experimental values gives the parameters k_2/k_1 and K listed in Table 1.

$$\frac{k(obs.)}{k_1[OH^-]} = \frac{1+(k_2/k_1)K[C]}{1+K[C]]} \qquad (2)$$

The equilibrium constants, K, calculated from the kinetic data correlate satisfactorily with those determined by distribution measurements[22] for the heteroassociation between free purine and the cosolutes. The values of k_2/k_1 referring to neutral cosolutes are all of approximately the same magnitude, ranging from 0.6 to 0.8. Accordingly, association of the starting material with another heteroaromatic molecule reduces its susceptibility to the nucleophilic attack of hydroxide ion. This is understandable on the basis of the structure of the transition state. Due to the attack of the hydroxide ion, the C8 atom of the purine ring becomes tetrahedrally bonded, or at least it may be expected to resemble a sp^3 hybridized carbon atom. Consequently, the polarizability of the π-electron system of the aromatic ring is reduced. Since the stacking-ability is known to correlate with the ring polarizability,[16] one may assume that the stacking interactions stabilize the initial state to a larger extent than the transition state. Accordingly, the decomposition of the substrate is retarded. The magnitude of the retardation may be more sensitive to the structure of the reacting molecule than to that of the cosolute.

Experimental. Materials. Adenine, purine, pyrimidine and pyridines were commercial reagents, and they were employed as received. The preparation of 1-(1-ethoxyethyl)cytosine,[25] 1-methyl- and 1,2-dimethylbenzimidazoles,[26] 9-methyl- and 8,9-dimethyladenines,[22] and 6-dimethylamino-9-ethoxymethylpurine[22] has been presented earlier. 9-(1-Ethoxyethyl)purine labeled with ^3H in the ethoxy group was synthesized by the method described for the unlabeled compound.[27] The isotopically modified ethyl vinyl ethyl, employed as starting material, was prepared from commercial ethyl vinyl ether and tritiated ethanol by mercury(II) acetate catalyzed vinyl transetherification.[28]

Kinetic measurements. The hydrolyses of the ^3H-labeled 9-(1-ethoxyethyl)purine were carried out in stoppered bottles immersed in a water bath, the temperature of which was kept constant within 0.1 K. The reactions were started by adding the substrate to the pre-thermostated alkaline cosolute solutions to give the concentration of 2×10^{-3} mol dm^{-3}. Aliquots of 2 cm^3 were withdrawn during 3 half-lives and transferred to funnels containing aqueous sodium dihydrogen phosphate to stop the reaction. The unreacted starting material was extracted in dichloromethane. The organic solvent was removed under reduced pressure, and the residues were transferred with water to liquid scintillation vials. The amount of the starting material present in the aliquots was determined by measuring the radioactivities on a LKB 81000 scintillation counter in lumagel.

UV-spectroscopic measurements indicated that the cosolutes did not undergo ionization or decomposition under the conditions employed in the kinetic studies.

Distribution measurements. The equilibrium constants for the association of pyrimidine and methyl substituted benzimidazoles with purine in aqueous solution at 298.2 K were determined by the distribution technique described previously.[22]

Acknowledgements. The financial aid from the Academy of Finland, Council for the Natural Sciences, is gratefully acknowledged.

1. Part XIV of the series "Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues". Lönnberg, H. and Lehikoinen, P. *J. Org. Chem. 49* (1984) 4964. Part XIII.
2. Helmkamp, G.K. and Kondo, N.S. *Biochim. Biophys. Acta 157* (1968) 242.
3. Nakano, N.I. and Igarashi, S.J. *Biochemistry 9* (1970) 577.
4. Schimmak, W., Sapper, H. and Lohman, W. *Biophys. Struct. Mechanism 1* (1975) 311.
5. Plesiewicz, E., Stepien, E., Bolewska, K. and Wierzchowski, K.L. *Biophys. Chem. 4* (1976) 131.
6. Lönnberg, H., Ylikoski, J. and Vesala, A. *J. Chem. Soc. Faraday Trans. 1, 80* (1984) 2439.
7. Ts'O, P.O.P., Melvin, I.S. and Olson, A.C. *J. Am. Chem. Soc. 85* (1963) 1289.
8. Broom, A.D., Schweizer, M.P. and Ts'O, P.O.P. *J. Am. Chem. Soc. 89* (1967) 3612.
9. Gill, S.J., Downing, M. and Sheats, G.F. *Biochemistry 6* (1967) 272.
10. Solie, T.N. and Schellman, J.A. *J. Mol. Biol. 33* (1968) 61.
11. Plesiewicz, E., Stepien, E., Bolewska, K. and Wierzchowski, K.L. *Nucl. Acids Res. 3* (1976) 1295.
12. Neurohr, K.J. and Mantsh, H. *Can. J. Chem. 57* (1979) 1986.
13. Scheller, K.H., Hofstetter, F., Mitchell, P.R., Prijs, B. and Sigel, H. *J. Am. Chem. Soc. 103* (1981) 247.
14. Ts'O, P.O.P. and Chan, S.I. *J. Am. Chem. Soc. 86* (1964) 4176.
15. Garland, F. and Christian, S.D. *J. Phys. Chem. 79* (1975) 1247.
16. Lawaczek, R. and Wagner, K.G. *Biopolymers 13* (1974) 2003.
17. Crothers, D.M. and Ratner, D.I. *Biochemistry 7* (1968) 1823.
18. Bascones, A., Peral, F. and Gallego, E. *An. Quim. 77* (1981) 105.
19. Hevesi, L., Wolfson-Davidson, E., Nagy, J.B., Nagy, O.B. and Bruylants, A. *J. Am. Chem. Soc. 94* (1972) 4715.
20. Lönnberg, H., Lehikoinen, P. and Neuvonen, K. *Acta Chem. Scand. B 36* (1982) 707.
21. Lönnberg, H., Lukkari, J. and Lehikoinen, P. *Acta Chem. Scand. B 38* (1984) 573.
22. Lönnberg, H., Ylikoski, J., Arpalahti, J., Ottoila, E. and Vesala, A. *Acta Chem. Scand. A 39* (1985) 171.

23. Vesala, A. and Sahlström, P. *Finn. Chem. Lett.* (1984) 43.
24. Gordon, J.E. *The Organic Chemistry of Electrolyte Solutions,* Wiley, New York 1975, Chapter 1.
25. Lönnberg, H. *Tetrahedron 38* (1982) 1517.
26. Arpalahti, J. and Lönnberg, H. *Inorg. Chim. Acta 78* (1983) 63.
27. Lönnberg, H. *Acta Chem. Scand. A 34* (1980) 703.
28. Watanabe, W.H. and Conlon, L.E. *J. Am. Chem. Soc. 79* (1957) 2828.

Received December 4, 1984.