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INTRAMOLECULAR PARTICIPATION IN THE HYDROLYTIC DEAMINATION OF 1-β-D-ARABINOSYLCYTOSINE

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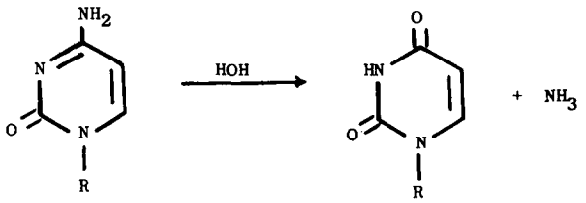
Cytosine (C), cytidine (Cyd) and 1-β-D-arabinosylcytosine (Ara-C) are known to undergo hydrolytic deamination in aqueous buffered solutions to yield the corresponding uracil derivatives as shown in Scheme I (1-3). Buffer catalysis by acetate, phosphate, lactate and several

Scheme I



R		
H	Cytosine (C)	Uracil (U)
ribose	Cytidine (Cyd)	Uridine (Urd)
arabinose	Arabinosylcytosine (Ara-C)	Arabinosyluracil (Ara-U)

other systems, has been demonstrated in all three cases and the catalytic mechanisms have been discussed (1-3). We have now completed a study which provides data for a direct comparison of the deamination rate constants for C, Cyd, and Ara-C in the absence of any catalytic effects by the buffers (see Table I). The values representing the first-order rate constants for deamination in the absence of buffer were obtained from the intercepts of plots of k_1 vs total buffer concentration at constant pH and ionic strength. Such plots are linear for Cyd and C but show curvature in the case of Ara-C. This difference in buffer effects will be discussed in a separate paper which deals with the details of buffer catalysis. The rate constants at pH 4.6, 70° in the absence of buffer for the deamination of Ara-C, Cyd, and C are 105, 2.58 and 1.89 (10^4 k_1 in $hr.^{-1}$). This represents a ratio of 56/1.4/1.0. Although a slight rate enhancement is noted when Cyd is compared to C, there is a 41 fold increase observed when Ara-C is compared

TABLE I

EXPERIMENTAL CONDITIONS[a] AND APPARENT FIRST-ORDER RATE CONSTANTS FOR HYDROLYTIC DEAMINATION OF ARABINOSYLCYTOSINE (Ara-C), CYTIDINE (Cyd) AND CYTOSINE (C) AT pH 4.60 ± 0.05[b]

	Buffer Compn.			$10^4 k_1$ (hr.$^{-1}$)		
°C	[CH_3COOH]	[CH_3COONa]	[NaCl]	Ara-C		Cyd
70°	0.36	0.36	0.00	267		7.67
	0.10	0.10	0.26	180		3.91
	0.050	0.050	0.31	151		3.28
	0.005	0.005	0.35	111		2.66
	0.00	0.00	0.36	105[c]		2.58[c]
80°	0.32	0.32	0.00	468		18.0
	0.090	0.090	0.23	306		9.92
	0.045	0.045	0.27	249		8.17
	0.005	0.005	0.31	179		6.46
	0.00	0.00	0.32	170[c]		6.42[c]
					C	
70°	0.20	0.20	0.00		3.59	
	0.15	0.15	0.05		3.16	
	0.082	0.082	0.12		2.54	
	0.020	0.020	0.18		2.07	
	0.00	0.00	0.20		1.89[c]	

[a] Assay methods for Ara-C, Cyd and C are reported elsewhere (1-3).

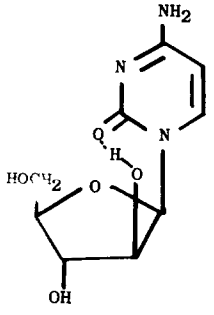
[b] The initial concentrations of nucleosides and the ionic strengths of the buffers were: 5.0×10^{-4} M., 0.32 or 0.36 for both Ara-C and Cyd; 1.8×10^{-3} M. 0.20 for C.

[c] Determined from the intercept of k_1 vs total buffer concentration.

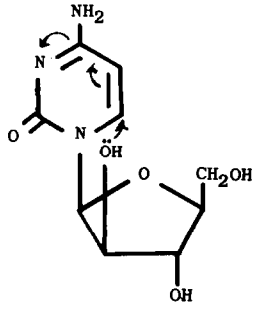
to Cyd. Since these rate constants do not reflect differences in the intermolecular catalysis by buffer, it is apparent that the 2'-hydroxyl in the arabinosyl nucleoside is enhancing the deamination rate through intramolecular catalysis. The rate constants for Ara-C and Cyd at 70° and 80° have been used to calculate the parameters in the Arrhenius expression. The energy of activation (Ea in Kcal/mole) and log A terms are: 11.6, 5.401 for Ara-C and 22.0, 10.458 for

Cyd. Thus the ratio of the deamination rate constants, Ara-C/Cyd, increases with decrease in temperature. We have calculated the rate constants at room temperature (25°) to be ($10^6 k_1$ in $hr.^{-1}$) 796 for Ara-C and 2.10 for Cyd yielding a Ara-C/Cyd ratio of 380/1.

This participation by the 2'-hydroxyl in the case of Ara-C, may be due to intramolecular catalysis by either a general-acid or a general-base mechanism. It is thought that dissociation of the 2'-hydroxyl in arabinosyl compounds affects the pyrimidine ultraviolet chromophore (4). Arabinosylnucleosides give markedly greater spectral shifts in alkaline regions compared to the ribosyl or xylosyl compounds. This has been attributed to rupture of the hydrogen bonding between the 2'-hydroxyl and the 2-carbonyl (Scheme II). Participation through the hydrogen-



General Acid



General Base

Scheme II

bond mechanism would constitute general-acid catalysis. Scheme II also illustrates a possible mechanism for general-base catalysis by nucleophilic attack of the 2'-oxygen on the C_6 position of the pyrimidine. This intramolecular general-base attack at C_6 is similar to the mechanism previously discussed for intermolecular catalysis by buffers (1, 2). Molecular models can be constructed to illustrate either type of ring formation. However, the latter is more likely since it represents formation of a 5-membered ring resembling that of the 6,2'-anhydronucleoside formed from 1-β-D-arabinofuranosyl-5-fluorouracil by intramolecular nucleophilic attack by the 2'-hydroxyl anion in 0.1 N NaOH at 60-70° (5).

A comparison of the catalytic constants for the deamination of C, Cyd, and Ara-C by various buffer species should aid in elucidating the mode of intramolecular catalysis in the Ara-C case. An extensive study of buffer catalysis and mechanisms is near completion and will be published in detail shortly in another journal (6).

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(6) This work has since been completed and a manuscript, by the authors of this letter, submitted to J. Pharm. Sci. Of significance to the current communication is the fact that general-acid catalysis clearly predominates in the deamination of Ara-C whereas deamination of C and Cyd is catalyzed by both acidic and basic buffer components. This implies that Ara-C provides its own general-base via intramolecular participation by the 2'-hydroxyl. Thus only a source of protons is required for Ara-C deamination to proceed. This further supports the general-base mechanism proposed in Scheme II.

Acknowledgement

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