DEAMINASE ACTIVITY OF COMMERCIAL SPLEEN PHOSPHODIESTERASE*

Kelvin K. Ogilvie and Robert L. Letsinger

Department of Chemistry
Northwestern University
Evanston, Illinois

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Commercially available spleen phosphodiesterase converts adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. It does not affect adenine or the other common nucleosides. Knowledge of this reaction is important for proper evaluation of structural data based on degradation of oligonucleotides by spleen phosphodiesterase.

Materials and Methods. Spleen phosphodiesterase was obtained as a lyophilized powder from Nutritional Biochemicals Corp. (15-20 units per vial) and from Worthington Biochemical Corp. (20 units per vial). Both samples gave the same results with all compounds tested. The "standard conditions" for the enzymatic reactions were the following (compare Ralph et. al., 1963; Razzell and Khorana, 1961): To 0.1-0.75 mg of

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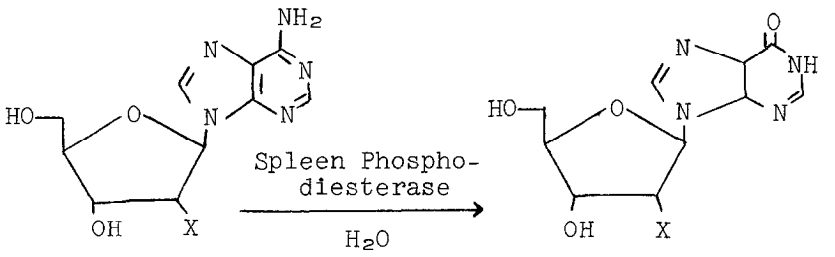
substrate in 0.2 ml of acetate buffer at pH 6.5 was added 1.5-2.0 units of the spleen phosphodiesterase in 0.1 ml of pyrophosphate buffer at pH 6.5. After incubation at 37° for 5 hr the solution was applied to Whatman 3MM chromatography paper and chromatographed with solvent A (i-propyl alcohol:ammonium hydroxide:water in vol. ratio 7:1:2), F (n-propyl alcohol:ammonium hydroxide: water in vol. ratio 55:10:35) or n-butyl alcohol:water (86:14 v/v) by the descending technique. Ultraviolet spectra of the products were obtained by cutting the nucleotide bands from the dried papers, eluting with 0.01 M hydrochloric acid, and measuring the spectra on a Cary 11 recording spectrophotometer.

Results and Discussion. Deamination of an adenine nucleoside by spleen phosphodiesterase was first observed in this laboratory in the course of characterizing a sample of thymidylyl-(3'-5')-deoxyadenosine that had been synthesized by the phosphotriester method (procedure of Letsinger and Ogilvie, 1967). This dinucleoside monophosphate yielded thymidine and deoxyadenosine-5' phosphate as expected when treated with snake venom phosphodiesterase. When treated with spleen phosphodiesterase it was similarly converted cleanly to two products. One was the expected thymidine-3' phosphate (R_f 0.44 in solvent F; 1.75 OD_{260} units). The other(R_f 0.67 in solvent F; 2.45 OD_{260} units) moved somewhat more slowly than deoxyadenosine (R_f 0.75) when chromatographed with solvent F. A comparison with other nucleosides revealed that this product was in fact deoxyinosine.



X = H or OH

TABLE I

Pertinent Chromatographic and Spectral Properties

Nucleoside	R_f in Solvent		Spectra in 0.01 M HCl	
	A	F	λ_{max}	λ_{min}
Adenosine	0.51	0.72	257	229
Inosine	0.35	0.62	248	222
Deoxyadenosine	0.60	0.75	257	232
Deoxyinosine	0.41	0.67	249	228

It was then found that adenosine and deoxyadenosine are converted quantitatively to inosine and deoxyinosine, respectively, by the spleen phosphodiesterase preparations under the "standard conditions" employed for cleaving phosphodiester bonds. Adenine, cytidine, deoxycytidine, guanosine, and deoxyguanosine were not affected by this enzyme under these conditions. Data used in characterizing the products derived from the adenine nucleosides are summarized in Table I. The specificity of the enzyme resembles that for the adenosine deaminases isolated from calf intestinal mucosa

and rat heart muscle (Kalckar, 1947; Brady and O'Connell, 1962; Chilson and Fisher, 1963; Bar and Drummond, 1966).

Adenine nucleoside monophosphates were found to react slowly in the presence of the commercial enzyme preparations of spleen phosphodiesterase to give the corresponding hypoxanthine nucleosides. Thus, deoxyadenosine-5' phosphate was partially (38%) converted to deoxyinosine by spleen phosphodiesterase under the standard conditions. The remainder of the deoxyadenosine-5' phosphate was recovered unchanged. A similar experiment with adenylic acid (Nutritional Biochemicals Corp.) yielded inosine (21%) and adenylic acid (79%). A plausible explanation for this behavior is that the spleen enzyme contains a small amount of a phosphomonoesterase which slowly dephosphorylates adenine nucleotides; subsequent deamination of the liberated nucleoside would then afford the observed hypoxanthine derivative.

Since spleen phosphodiesterase has been used extensively to degrade oligonucleotides for sequences studies and structural proofs (Hilmoe, 1960; Smith et al., 1962; Hayatsu and Khorana, 1967) the observation that adenosine and deoxyadenosine are deaminated by commercial preparations of this enzyme is noteworthy. Clearly, cognizance of this reaction should be taken in evaluating data for hydrolytic reactions catalyzed by the spleen enzyme.

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