XANTHINE OXIDASE AND ADENOSINE DEAMINASE IN COMMERCIAL BOVINE SPLEEN PHOSPHODIESTERASE PREPARATIONS

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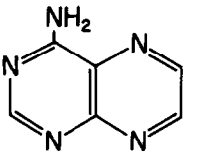
Received February 27,1975

Summary Commercial bovine spleen phosphodiesterase preparations contain xanthine oxidase activity; the xanthine oxidase in such preparations mediates the oxidation of a pteridine derivative as well as a standard purine substrate (hypoxanthine). The xanthine oxidase activity in the phosphodiesterase preparations is inhibited strongly by allopurinol (4-hydroxypyrazolo(3,4-d) pyrimidine). The reported ability of phosphodiesterase preparations to catalyze the deamination of adenosine derivatives appears to be due to contamination with a conventional adenosine deaminase in view of the observations that this activity is inhibited by an established inhibitor of adenosine deaminase and that the relative rates of deamination of N^1-methyladenosine and adenosine are similar with both the phosphodiesterase preparation and calf intestine adenosine deaminase.

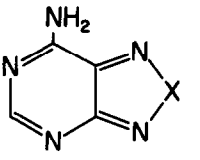
INTRODUCTION

Preparations of bovine spleen phosphodiesterase have been reported recently (1) to be capable of mediating the deamination of several 6-aminopurine nucleosides (e.g., adenosine, deoxyadenosine and N^6-methyldeoxyadenosine). It should be stressed that this is not the first report of "aminopurine nucleoside deaminase" activity in phosphodiesterase preparations since Ogilvie and Letsinger (2) described, in 1968, the ability of phosphodiesterase preparations to deaminate both adenosine and deoxyadenosine. Certain pteridines and related fused pyrimidine derivatives lacking a sugar substituent, have been found to be substrates for adenosine deaminase (3,4). Because of interest in this laboratory

in the chemistry and biochemistry of pteridines and related heterocyclic systems we have been investigating the interactions of such compounds with adenosine deaminase. We wished to determine whether the deaminase activity observable in phosphodiesterase was capable of mediating deamination of 4-aminopteridine (I) and the formally related compounds 7-aminothiadiazolo(3,4-d)pyrimidine (II) and 7-aminofurazano(3,4-d)pyrimidine (III), since each of these compounds has been found to be deaminated by adenosine deaminase from both a mammalian and a fungal source (4). We also wished to determine whether the deaminase activity present in phosphodiesterase preparations is subject to inhibition by agents which inhibit adenosine deaminase from calf intestine. This communication presents evidence suggesting that the deaminase activity present in phosphodiesterase is, in fact, adenosine deaminase; the occurrence of xanthine oxidase activity in phosphodiesterase preparations also is reported.



I.



II. X=S
III. X=O

MATERIALS AND METHODS

Deoxyadenosine was purchased from California Corporation for Biochemical Research; Adenosine was purchased from Sigma Chemical Co.; Purine riboside, N^6-methylaminopurine riboside, N^1-methylaminopurine riboside, hypoxanthine and allopurinol were obtained from Aldrich Chemical Corp.; 4-aminopteridine and 4-hydroxypteridine were synthesized in this laboratory using a standard procedure (5); 7-aminothiadiazolo(3,4-d)pyrimidine and 7-aminofurazano(3,4-d)-pyrimidine were provided by Professor E. C. Taylor, Department of Chemistry,

Princeton University. Bovine spleen phosphodiesterase preparations were obtained from the Worthington Biochemical Corporation. Calf intestine adenosine deaminase was purchased from P-L Laboratories. Spectroscopic assessment of deaminase activity and xanthine oxidase activity were performed at 34±1° using a Perkin-Elmer model 200 recording spectrophotometer or at 37° using a Beckman model 25 kinetic spectrophotometric system. All enzymatic studies were done at pH 7.0 (0.1 M phosphate buffer) with substrates at 1×10^{-4}M and 50 µl of the phosphodiesterase preparation (10 units/ml) in a 1.0 ml system; inhibition studies were carried out by pre-incubating the enzyme preparation for 2 minutes with the appropriate inhibitor (1×10^{-4}M) before addition of the substrate.

RESULTS AND DISCUSSION

7-Aminothiadiazolo(3,4-d)pyrimidine (II; figure 1) was slowly converted by the phosphodiesterase preparation to a product with identical spectroscopic properties to those observed when II was incubated with calf intestinal adenosine deaminase. The ultraviolet maximum of II at 340 nm gradually disappeared and was replaced by a new maximum at 315 nm; the absorption maxima of the reaction product in acid solution (pH 1; 310 nm) and basic solution (pH 13; 337nm) coincided with those reported for the expected deamination product, 7-hydroxythiadiazolo(3,4-d)pyrimidine (6). When I and III were incubated with phosphodiesterase, the ultraviolet spectral changes were quite different from those observed with these compounds in the calf intestine adenosine deaminase system and, in fact, resembled quite closely those observed in earlier studies (7,8) of the oxidation of I and III by xanthine oxidase. We therefore examined the phosphodiesterase preparation for the presence of xanthine oxidase activity. As shown in Table I the phosphodiesterase preparation efficiently converts hypoxanthine to uric acid as measured by the increase in absorbancy at 290 nm; this conversion was inhibited completely by allopurinol, a potent inhibitor of xanthine oxidase (9). 4-Hydroxypteridine

TABLE I

Effects of Allopurinol and of Purine Riboside on Absorbancy Changes Mediated by PDE Preparations

Substrate	Analytical Wavelength	No Inhibitor	Inhibitor Added
	Allopurinol Studies		
Hypoxanthine	290	0.140/30 min	0.010/30 min
4-Hydroxypteridine	330	0.160/60 min	0.005/30 min
I	320	0.060/120 min	0.010/120 min
II	340	0.100/60 min	0.100/60 min
III	340	0.110/60 min	0.010/60 min
	Purine Riboside Studies		
Adenosine	260	0.160/2 min	0.040/2 min
II	340	0.080/60 min	0.010/60 min
III	340	0.090/60 min	0.080/60 min

All experiments (except those with adenosine) were performed using pH 7.0 phosphate buffer (0.1M; 0.85 ml), 50 μl of the phosphodiesterase preparation (concentration in reaction solution was 0.05 unit/ml) and 0.1 ml of the appropriate substrate solution (substrate concentration in the reaction solution was 1.0×10^{-4}M). The studies with adenosine were performed with a concentration of phosphodiesterase in the reaction solution of 0.005 unit/ml. Inhibition studies were carried out by adding either allopurinol (1×10^{-4}M) or purine riboside (1×10^{-4}M) to the enzyme solution prior to addition of the appropriate substrate.

is a good substrate for xanthine oxidase from bovine milk (10) and mammalian liver (11); the phosphodiesterase preparation also converted 4-hydroxypteridine to a product identified spectroscopically as 2,4,7-trihydroxypteridine, the same product observed when 4-hydroxypteridine is oxidized by xanthine oxidase from milk or mammalian liver; the oxidation of 4-hydroxypteridine by the spleen phosphodiesterase was suppressed strongly by allopurinol (Table 1).

We next examined the spectroscopic changes characteristic of the alteration

of I, II and III in the phosphodiesterase system, in the presence of sufficient allopurinol to suppress the contribution of xanthine oxidase mediated reactions to such spectroscopic changes. The pattern of spectroscopic changes observed when I is incubated overnight with the phosphodiesterase preparation, in the presence of allopurinol, was quite different from that observed in the absence of this xanthine oxidase inhibitor. In the phosphodiesterase system without allopurinol the appearance of a sharp maximum at 326 nm and a shoulder at 338 nm characterized the reaction while in the presence of allopurinol these changes were not observed but rather the reaction was characterized by the appearance of a new peak at 314 nm. The spectroscopic changes observed with (I) in the phosphodiesterase system, containing allopurinol, were comparable to those observed for deamination of (I) mediated by calf intestine adenosine deaminase. The rapid spectroscopic changes observed when compound (III) was incubated with phosphodiesterase were inhibited virtually completely by allopurinol, indicating that such changes were mainly attributable to the action of xanthine oxidase in the preparation. Compound II when incubated with phosphodiesterase in the presence of allopurinol, underwent spectroscopic changes essentially identical to those observed in the absence of allopurinol and to those observed when (II) is deaminated by calf intestine adenosine deaminase. The experiments with allopurinol therefore suggest that the spectroscopic changes seen with I and III in the phosphodiesterase system are largely due to the presence of a xanthine-oxidase contaminant in the preparation whereas the changes observed with II are due predominantly to the presence of adenosine deaminase in the preparation. That the adenosine deaminase activity present in the phosphodiesterase preparations is of a conventional type is indicated by our observations that an inhibitor of a standard adenosine deaminase preparation (calf intestine) produces comparable inhibition of the adenosine deaminase activity in the phosphodiesterase preparation;

as shown in table (I) purine riboside, an established inhibitor of adenosine deaminase (12) suppresses the spectroscopic changes observed when either adenosine or II is incubated within the phosphodiesterase preparation. It is also worthy of mention that we found the deaminase contaminant present in phosphodiesterase to deaminate N^1-methyladenosine at a rate approximately 1000 times less than that observed for adenosine when both substrates were evaluated at a concentration of 1.5×10^{-4}M; these relative velocities of deamination in the phosphodiesterase system are comparable to those reported for calf intestinal adenosine deaminase by Wolfenden and his colleagues (4) but considerably different from reported by other investigators (13). Our studies of the relative susceptibilities of N^1-methyladenosine and adenosine to deamination by calf intestine adenosine deaminase were in substantial agreement with the studies reported by Wolfenden.

ACKNOWLEDGEMENTS - This work was supported, in part, by USPHS (NCI) Grant CA 12876. Thanks are due to Mrs. Ann Falivene for excellent technical assistance.

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