IN VITRO INHIBITION OF CATALASE BY 4-HYDROXYPYRAZOLE, A METABOLITE OF PYRAZOLE

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1. Introduction

Pyrazole is an effective inhibitor of catalase (EC 1.11.1.6) when given in vivo, but not *in vitro* [1,2]. It has been suggested [3] that the *in vivo* inhibition is mediated by 'a product from the metabolism of pyrazole by the microsomal mixed-function oxidase system. . .in a process which can be prevented by alcohol.' From a study of the action of pyrazole on tryptophan oxygenase (EC 1.13.1.12), another hemoprotein enzyme, it has also been proposed that the delayed in vivo inhibition seen after pyrazole treatment, much stronger than any comparable in vitro inhibition, results from either an inhibitory action on tryptophan oxygenase synthesis and/or a direct interference with the heme prosthetic group by an active derivative of pyrazole [4].

Work in our laboratory [5] has revealed that the major metabolite of pyrazole in rats is 4-hydroxy-pyrazole, excreted in hydrolyzable conjugated forms. Production of this metabolite is inhibited by ethanol. We now demonstrate that 4-hydroxypyrazole is a good inhibitor of catalase in vitro.

2. Materials and methods

3-Amino-1,2,4-triazole was purchased from Nutritional Biochemicals (Cleveland, USA). 4-Hydroxy-pyrazole and 4-aminopyrazole were gifts from Lilly Research Laboratories (Indianapolis, USA). Pyrazole was purchased from Aldrich (Milwaukee, USA), and recrystallized from petroleum ether. Recrystallized beef liver catalase was purchased from Worthington (Freehold, NJ, USA).

Catalase activity was determined by a slight modification of the Worthington [6] version of the assay of Beers [7]. The enzyme was diluted 1:1000 with 0.05 M phosphate buffer, pH 7. At zero time, 0.02 ml of this solution was mixed into a 3 ml (10 mm) quartz cuvette by the hanging-drop method of Orr [8]. When inhibitors were tested, they were added to the buffer and hydrogen peroxide in the cuvette a few minutes prior to the addition of the enzyme. The absorbance at 240 nm was then followed for 2 min using a Beckman DB-GT recording spectrophotometer.

3. Results

The effects of various concentrations of pyrazole, 3-aminotriazole, 4-aminopyrazole, and 4-hydroxy-pyrazole are shown in table 1. Pyrazole has no effect on catalase in vitro, as previous papers [2,3,9] have pointed out. 4-Aminopyrazole is a good inhibitor of catalase in vitro, even with no pre-incubation with the enzyme. Feinstein et al. [10] had shown that 4-aminopyrazole is a potent inhibitor in vivo and in his pre-incubated system in vitro, with a concentration for 50% inhibition of 3.8 mM. As expected [11], 3-aminotriazole is a weak catalase inhibitor in our un-incubated system, a concentration of 40.0 mM being required for 50% inhibition; whereas in Feinstein's pre-incubated system, only 5.3 mM is necessary. Our result with 3-aminotriazole corresponds more closely with the result obtained by Heim [12] of

Table 1
Percent inhibition of the catalatic reaction of catalase in vitro by various concentrations of inhibitors

Concentration (mM)	1.0	1.5	2.0	3.0	4.0	5.0
4-Aminopyrazole	40%	61%	73%	–	–	–
	32%	61%	77%			
4-Hydroxypyrazole	8%	–	32%	44%	56%	62%
	11%		32%	47%	54%	68%
	30.0	40.0	50.0			
3-Aminotriazole	34%	50%	63%			
	37%	51%	60%			
Pyrazole	–	–	0%			

Rate with no inhibitor present was −0.0293 ± 0.0006 (se) A/min, corresponding to about 2 units of enzyme/cuvette. Half inhibition concentration was obtained from a plot of percent inhibition versus log concentration of inhibitor. Each value represents one determination.

'slightly in excess of 0.05 M' needed for half inhibition in vitro.

4-Hydroxypyrazole is a much more potent inhibitor than 3-aminotriazole in our system, with 3.38 mM needed for half inhibition, a slightly weaker inhibition than that produced by 4-aminopyrazole. All three inhibitors are capable of producing 100% inhibition of catalase in vitro. Ethanol (54 mM) does not affect 4-hydroxypyrazole's inhibition of catalase.

4. Discussion

These results, and our previous demonstration of the fact that ethanol almost completely inhibits metabolism of pyrazole to 4-hydroxypyrazole, indicate that 4-hydroxypyrazole is the active metabolite of pyrazole inhibiting catalase in vivo. This direct demonstration obviates the need (1) to postulate an interference by ethanol with binding of 4-hydroxypyrazole to catalase [3], or (2) to invoke an inhibition of protein synthesis for the inhibition observed after administration of pyrazole in vivo [4,13] in the case of catalase.

All of the most active compounds tested by Feinstein [10] contained the N=C(X)–N structure, were X = NH_2 or SH, except for 4-aminopyrazole. 4-Aminopyrazole, although very active as a catalase inhibitor, is the only compound listed in this category by Feinstein which does not fit the rules for 'irreversible catalase inhibitors' laid down by Margoliash [11], with Feinstein's revision that the primary amine group may be replaced by a thiol group. Hence it would not be surprising if 4-aminopyrazole, and its close analogue 4-hydroxypyrazole, inhibited catalase via a different mechanism from that of 3-aminotriazole and its analogues. In fact, 4-aminopyrazole does differ from 3-aminotriazole in that the inhibition is as strong immediately after addition to catalase as it is after incubation with catalase and a source of peroxide [10]. Inhibition by 4-hydroxypyrazole also appears to take effect immediately.

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