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Irreversible inactivation of catalase by 3-amino-1,2,4-triazole*

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Studies published over a quarter of a century ago [1, 2] demonstrated that 3-amino-1,2,4-triazole (3-AT) does not inhibit catalase, except in the simultaneous presence of H_2O_2. These workers showed that a low, but constant, level of H_2O_2 is essential for this inactivation, which, once effected, is not reversed by extensive dialysis. They also showed, using ^{14}C-labeled 3-AT, that inactivation is associated with the binding of one molecule of 3-AT per hematin prosthetic group and that this covalently bound 3-AT is associated with the catalase protein, rather than with the hematin prosthetic group. Indeed, Chang and Schroeder [3] subsequently isolated and sequenced the tryptic peptide to which the radiolabeled 3-AT was attached.

Recently, Williams *et al.* [4] reported that purified catalase is not inactivated by 100 μg/ml of 3-AT, even when exposed for 4 hr at 37°. Moreover they failed to see inhibition when H_2O_2 was added to the buffer to a final concentration of 1 mM. Since the catalase in a crude homogenate of liver is gradually and irreversibly inactivated by 3-AT, under these conditions, they concluded that 3-AT was not itself able to inactivate catalase, but that some metabolite of 3-AT was able to do so. They dismissed the results of earlier workers [1, 2] on the grounds that their assay for catalase activity was less specific than the polarographic method now available.

Williams *et al.* [4] failed to provide for a continuous source of H_2O_2. Catalase, at the levels they used, added to 1.0 mM H_2O_2, will rapidly decompose it, such that none is available, after a few seconds of reaction, to support the inactivation of the enzyme by 3-AT. Crude homogenates of liver contain endogenous sources of H_2O_2, such that inhibition of catalase by 3-AT can occur in the absence of an exogenous source of this peroxide. Indeed, the inactivation of catalase within cells by 3-AT has been used as a measure of the rate of endogenous production of H_2O_2 [5, 6].

We deemed it essential to correct the misimpression created by Williams *et al.* [4], such that workers, new to the study of catalase, not be misled. To that end we examined the effect of 3-AT upon pure catalase, using dialysis against stirred, buffered, H_2O_2, to provide for a continuous infusion of H_2O_2 to replace that decomposed by the catalase. As expected from earlier work [1, 2], we observed a gradual, essentially complete, irreversible inhibition of catalase, by 3-AT, under these conditions.

Materials and methods

Bovine liver catalase was obtained as a crystalline suspension in water from the Sigma Chemical Co. Its concentration was based upon a molar extinction coefficient of $300 \times 10^3\ M^{-1}\ cm^{-1}$ at 406 nm [7], and its activity was measured polarographically by a modification of the method described by del Rio *et al.* [8].

Results and discussion

Catalase, at 4 nM, was dialyzed against 50 mM potassium phosphate, 5 mM 3-AT, 5 mM H_2O_2, 0.1 mM EDTA, all at pH 7.8 and at 22°, with stirring. At intervals, 4-μl aliquots were removed and were diluted into 2.0 ml of 50 mM potassium phosphate, 20 mM H_2O_2, 0.1 mM EDTA, at pH 7.8 and 25°, under a Clarke electrode. Residual catalatic activity was thus measured in terms of the initial rate of dioxygen evolution under these conditions. The results in Fig. 1 demonstrate that catalase, dialyzed against a mixture of 5.0 mM 3-AT plus 5.0 mM H_2O_2 (line 4), lost activity more rapidly than did catalase dialyzed against H_2O_2 alone (line 3) or 3-AT alone (line 2). Since residual catalase activity was assayed after a 500-fold dilution into an assay mixture free of 3-AT, we conclude that the inhibition imposed by 3-AT plus H_2O_2 was not rapidly reversible.

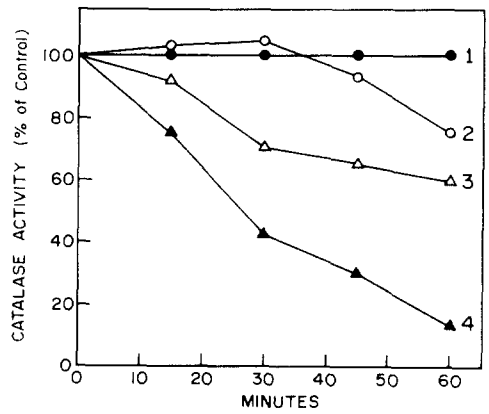

Fig. 1. Inactivation of catalase by 3-AT plus H_2O_2. Crystalline bovine liver catalase, at 4 nM, was dialyzed against 1000 vol. of 50 mM potassium phosphate, 5 mM H_2O_2 and 0.1 mM EDTA, at pH 7.8 and at 22°. At intervals samples were taken for assay of residual catalase activity. Additional components of the dialysis solution were: line 1, none; line 2, 5.0 mM 3-AT; line 3, 5.0 mM H_2O_2; and line 4, 5.0 mM 3-AT plus 5.0 mM H_2O_2.

It is clear from the data in Fig. 1 and from the pertinent earlier literature [1–3] that the results reported by Williams *et al.* [4] must be dismissed as artifactual and due to their failure to provide for a continuous supply of H_2O_2 during exposure of the catalase to 3-AT.

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