INACTIVATION OF CATALASE BY CHLORIDE

William J. LITCHFIELD
Johnson Research Foundation, University of Pennsylvania, Philadelphia, PA 19174, USA

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1. Introduction

A variety of acids react reversibly with catalase (EC 1.11.1.6) to yield spectroscopically distinct and inactive compounds [1–4]. These reactions are all second order, involve the undissociated form of the ligand, and generally do not require heme–heme interactions [3,4]. Reactions of catalase with cyanide, azide, formate, acetate, and fluoride have been extensively studied [1–4]. However, there is no previous information concerning the reaction of catalase with chloride.

In this paper, the activity of bovine liver catalase is shown to be significantly inhibited at low pH in the presence of chloride. This inactivation results from formation of a stable catalase–chloride compound (K_d 9.0 mM, at pH 4.4) with a stoichiometry of one chloride per catalase heme. Binding of chloride to catalase, as determined by difference spectrophotometry ($\Delta E^{405-390nm}_{mM^{-1}}$ 2.25), does not involve heme–heme interactions and requires the undissociated form of HCl. This reaction may commonly occur when catalase is present in physiological solutions at low pH.

2. Materials and methods

Lyophillized, thymol free catalase from bovine liver (Sigma Chemical Co., St Louis, MO) was initially diluted to a concentration of 0.83 μg/ml in 0.1 M potassium phosphate buffer of the appropriate pH (fig.1). Catalase activity at room temperature was measured immediately after adding 10 μmol H_2O_2 (Mallinckrodt, St Louis, MO) by recording the loss of absorbance at 230 nm. Catalase activity in the presence of chloride was determined in the same manner (fig.1, fig.2). However, data in fig.2 were plotted as described by Chance [5] where R_I and R_U represent the activities of enzyme in the presence and absence of chloride respectively.

All assays of enzyme activity and difference spectra were performed using either a Perkin-Elmer 356 or a Cary 15 double-beam spectrophotometer. Data in fig.5 were obtained with a dual wavelength multichannel spectrophotometer designed and built at the Johnson Research Foundation [6]. Catalase concentrations of between 4 μM and 5 μM were used in these binding experiments.

3. Results and discussion

The present study was begun to account for lower than expected catalase activity when this enzyme was assayed in Krebs Ringer phosphate solution (KRPS) at low pH. Since the buffer contained NaCl in excess of 150 mM [7], chloride concentration and ionic strength were first tested for effects on catalase function. In this regard, pH profiles of enzyme activity were run in the presence and absence of chloride (fig.1). The pH profile without chloride agreed with previously reported data on the pH dependence of catalase [8]. However, substantially different profiles were obtained when catalase was assayed in either KRPS or phosphate buffer containing 200 mM NaCl. Since the effect of NaCl was also observed by substituting 100 mM $MgCl_2$, but not 100 mM $(NH_4)_2SO_4$ (data not shown), general ionic strength interactions were ruled out as a cause of the catalase inhibition.

The relationship between total chloride concentration and catalase activity was further investigated

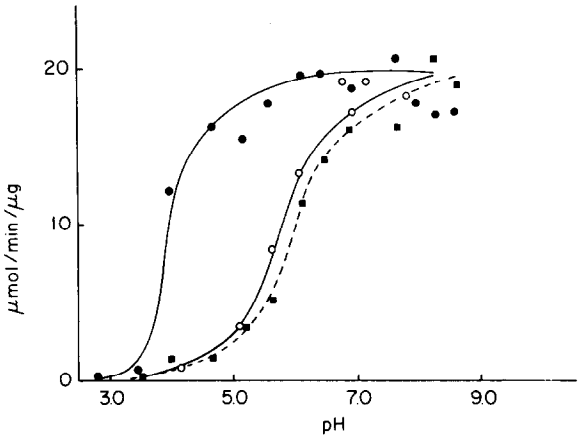

Fig.1. Specific activity of catalase as a function of pH at different chloride concentrations. Assays were performed in (•) 0.1 M potassium phosphate without chloride, (○) KRPS which contained 150 mM NaCl, and (▪) 0.1 M potassium phosphate plus 200 mM NaCl.

in phosphate buffer at pH 4.0 and pH 4.7. These results, when combined in fig.2, demonstrated that chloride concentration was inversely related to $R_I/(R_U - R_I)$. Therefore, both an apparent K_i

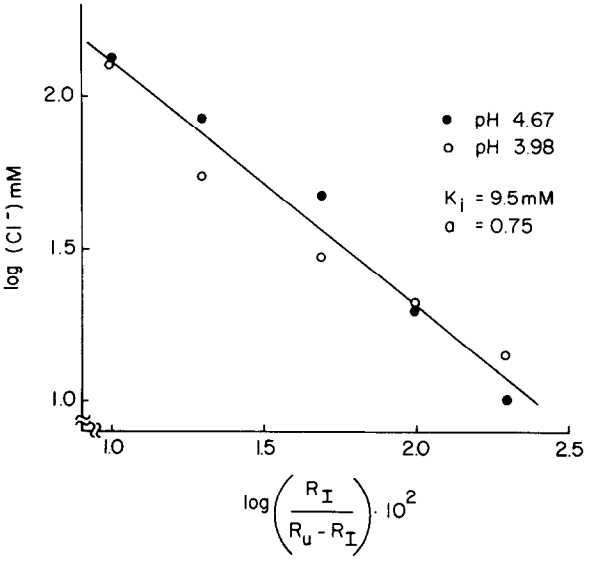

Fig.2. Relationship between total chloride concentration and catalase activity. R_I and R_U represent activities of enzyme in the presence and absence of chloride, respectively.

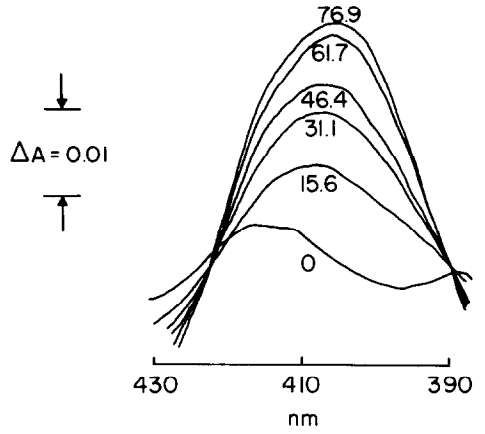

Fig.3. Difference spectra between catalase-chloride compound and free enzyme. Numbers near each trace indicate millimolar levels of chloride added.

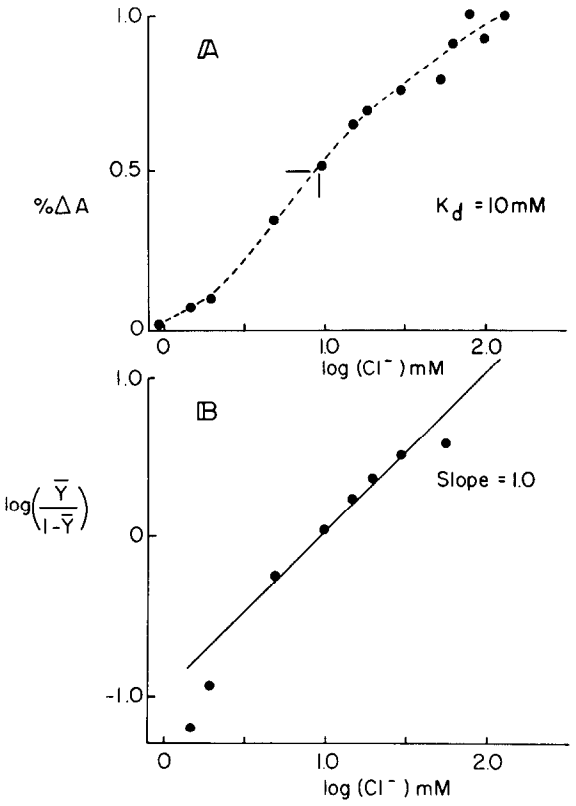

Fig.4. Perturbation of catalase absorption upon chloride binding. Both %A (in A) and $\bar{Y}$ (in B) represent normalized differences in absorbance at 405 nm minus 390 nm.

9.5 mM and a binding stoichiometry a of 0.75 chlorides/active site were calculated using the following equation [5]:

$$\log R_I/(R_U - R_I) = \log K_i - a\log(Cl^-)$$

Binding of chloride to catalase heme was verified by measuring the increase in Soret peak absorption of the catalase–chloride compound relative to the free enzyme (fig.3). Taking absorbance change between the maximum at 405 nm and the isosbestic point at 390 nm as an indicator of reaction, these data yielded a K_d of 10 mM for chloride at pH 4.4 (fig.4A). These data also indicated that little, if any, cooperativity occurred during the binding process (fig.4B), and subsequently, an extinction coefficient for the catalase-chloride compound minus free enzyme was calculated ($\Delta E^{405-390\ nm}_{mM^{-1}}$ 2.25). This value was employed along with a Scatchard type analysis [9] to determine a binding stoichiometry of 4 chlorides/catalase tetramer.

Binding affinity and stoichiometry were further investigated as a function of pH using dual wavelength difference spectrophotometry [6]. Data from these studies, presented in the Scatchard plots (fig.5A), indicated that the apparent pK_d chloride binding was inversely proportional to pH (fig.5B). This relationship was also found for the binding of other acids to catalase and was interpreted to mean that the protonated ligand, rather than conjugate base, was the actual reactive species [1,3,4]. Assuming the pK_a of HCl to be zero, binding of HCl to catalase would occur with a constant K_d of $(4.8 \pm 1.4) \times 10^{-7}$ M at these pH values (fig.5B).

Moreover, the above results should caution against the use of physiological buffers when determining catalase activity in the pH range 3.5–7.0. Practically all such buffers contain high isotonic levels of chloride and are commonly employed during cell and culture in vitro. In vivo conditions for catalase inhibition by chloride are probably quite rare. However, they might exist in specialized cells or tissues which contain both high levels of chloride and regions of

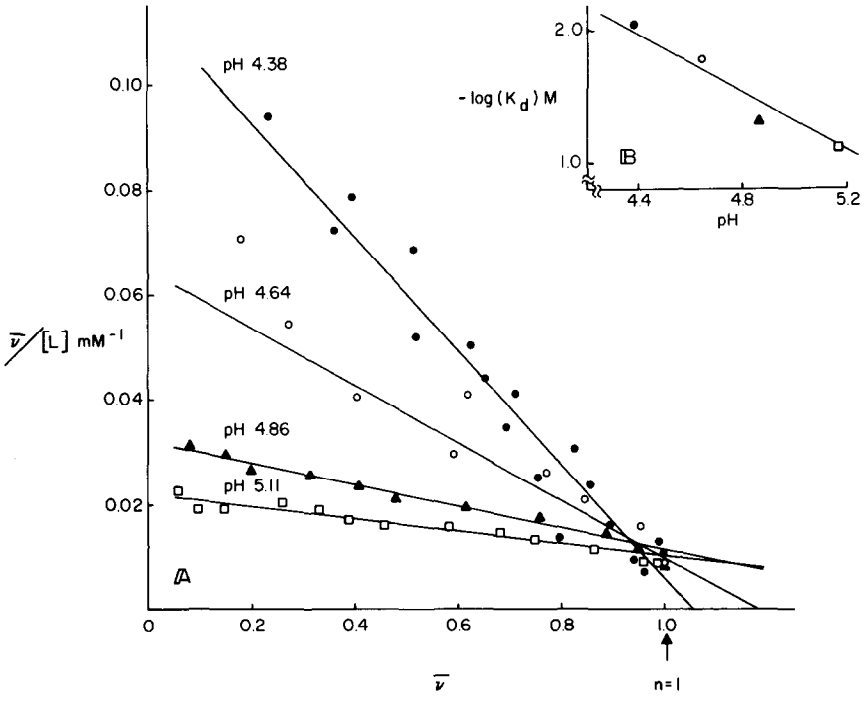

Fig.5. Binding of chloride to catalase at different pH values. Dissociation constants, derived from Scatchard plots (in A), are plotted verses pH (in B). L equals chloride concentration, and $\bar{\nu}$ represents the molar ratio of bound ligand to total catalase heme.

low intracellular pH. The polymorphonuclear leukocyte, which contains up to 120 mM chloride [10,11] and which has intravacuolar pH 3–5 [12,13], satisfies both of these criteria. Thus, leukocytes might conceivably inactivate endocytosed bacterial catalase through this mechanism. Such action would prevent competition between bacterial catalase and leukocytic myeloperoxidase for H_2O_2 and would increase the steady-state levels of H_2O_2 needed for antibacterial function of the myeloperoxidase–H_2O_2–chloride system [14–16].

Acknowledgements

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