PRELIMINARY NOTES

BBA 61127

Diamine oxidase inactivation by hydrogen peroxide

It is known that H_2O_2, which is produced during oxidation of diamines by diamine oxidase (diamine:O_2 oxidoreductase (deaminating), EC 1.4.3.6) (ref. 1), inactivates the enzyme[2-4]. MANN[3] demonstrated that pea seedling diamine oxidase was inactivated by H_2O_2 only when a substrate of the enzyme was present, suggesting that only the reduced form of the enzyme was affected by H_2O_2. Similar results were obtained with other enzymes, for example, with glucose oxidase (EC 1.1.3.4) as reported by KLEPPE[5]. In the present study, the inactivation by H_2O_2 of pig kidney diamine oxidase has been investigated in experiments which were particularly focused on the effect of pH on the inactivation.

Pig kidney diamine oxidase was purified according to MONDOVÌ *et al.*[6] as a protein homogeneous in the ultracentrifuge and by gel electrophoresis. The enzyme activity was followed by the O_2 uptake in a conventional Warburg apparatus at 38°. Air was used as the gas phase and cadaverine as the substrate. The total volume of the incubation mixture was 3 ml. The center well of the vessel contained 0.2 ml of 30% NaOH. The enzyme activity units are given for the standard conditions pre-

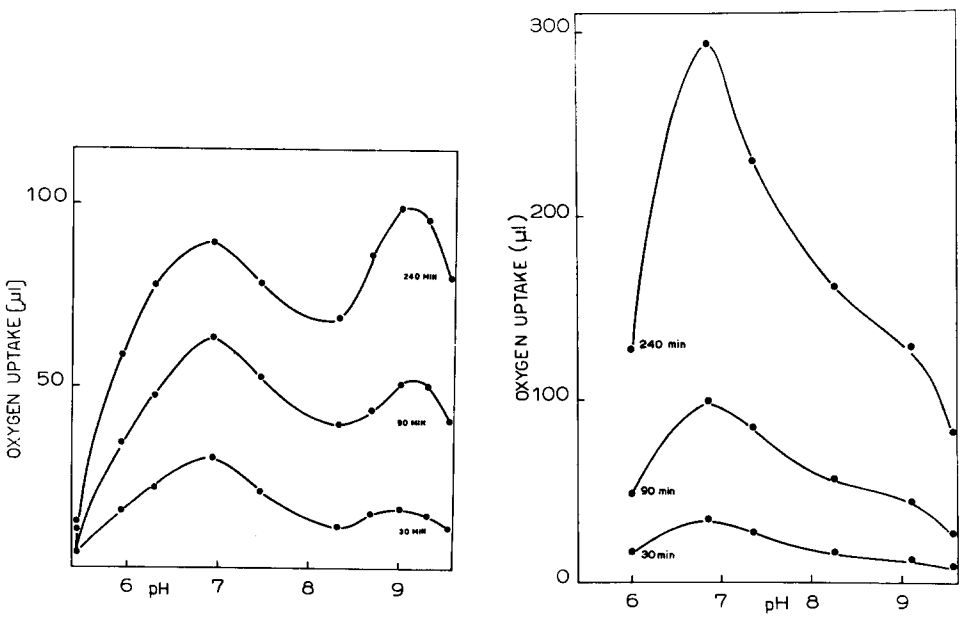

Fig. 1. Diamine oxidase activity as a function of pH. The reaction mixture, 3 ml total volume, contained diamine oxidase (0.03 units) and cadaverine (34 mM) as substrate. The buffers used were as follows. pH 5.4: 0.33 M acetate, pH 5.5–7.5: 0.1 M potassium phosphate, pH 8.3 and 8.7: 0.067 M borate–HCl, pH 9.3–9.55: 0.067 M borate–NaOH.

Fig. 2. Diamine oxidase activity as a function of pH. Reaction mixtures, 3 ml total volume, contained diamine oxidase (0.03 units), cadaverine (34 mM) as substrate, 100 µg of catalase and 34 mM ethanol. The buffers used were as described in Fig. 1.

viously reported[7], following the recommendations in the Report of the I.U.B. Enzyme Commission.

Diamine oxidase activity was analyzed in the pH range between 6 and 9.5 in the presence of catalase (EC 1.11.1.6) and ethanol and in the absence of these substances. The results obtained under these conditions would provide indications of the effect of H_2O_2 during the catalytic action.

Figs. 1 and 2 show that the rate of O_2 uptake is quite different under the two conditions: in the presence of catalase and ethanol the O_2 uptake is greatest at neutral pH and decreases on either side of this pH range. In the asbence of these substances, a double activity curve is evident: one at neutral pH and the other at alkaline pH.

The H_2O_2 produced during the enzymic oxidation of substrate may be measured by adding catalase at the end of the incubation. In this case controls are performed in the presence of catalase and ethanol. Fig. 3 shows that, at neutral pH, the O_2 liberated from H_2O_2 by the action of catalase added at the end of incubation corresponds only

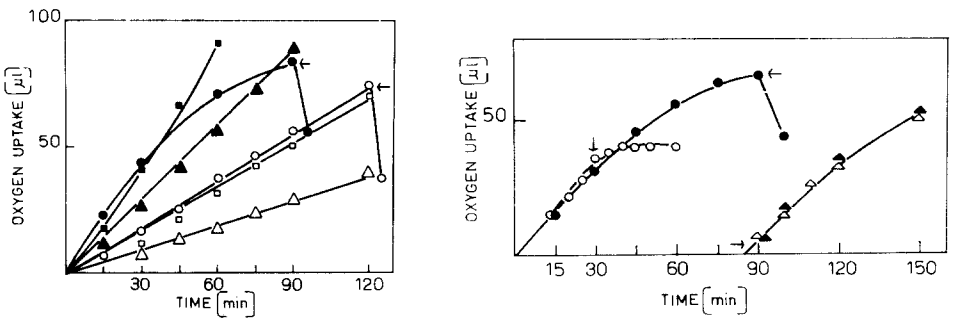

Fig. 3. Diamine oxidase activity at pH 7.4 and 9. Reaction mixtures, 3 ml total volume, contained diamine oxidase (0.06 units) and cadaverine (17 mM) as substrate. ■, incubation performed in 0.1 M potassium phosphate buffer (pH 7.4) in the presence of 100 µg of catalase and 34 mM ethanol; ●, incubation performed in 0.1 M potassium phosphate buffer (pH 7.4). 100 µg of catalase was added at the arrow; ▲, incubation performed in 0.1 M potassium phosphate buffer (pH 7.4) in the presence of 100 µg of catalase; □, incubation performed in 0.1 M borate buffer (pH 9) in the presence of 100 µg catalase and 34 mM ethanol; ○, incubation performed in 0.1 M borate buffer (pH 9). 100 µg of catalase was added at the arrow; △, incubation performed in 0.1 M borate buffer (pH 9) in the presence of 100 µg of catalase.

Fig. 4. Diamine oxidase activity in the presence of H_2O_2. Reaction mixture, 3 ml total volume, contained diamine oxidase (0.05 units) and cadaverine (17 mM) as substrate, in 0.1 M potassium phosphate buffer (pH 7.4). ●, incubation performed in the absence of catalase and ethanol. At the arrow, 100 µg of catalase was added; ○, incubation performed as above. At the arrow 2 mM H_2O_2 was added; ▲, control; the enzyme was pre-incubated as such (without cadaverine) at 38° for 80 min. At the arrow, the substrate was added; △, the enzyme was preincubated in the presence of 2 mM of H_2O_2. At the arrow, 100 µg of catalase was added. When H_2O_2 was completely destroyed, 17 mM cadaverine was added.

to a part of the H_2O_2 expected. On the other hand, at alkaline pH, the H_2O_2 produced corresponds exactly to the calculated value. In fact, the values of net O_2 consumption measured in the last case are the same whether incubations are performed in the presence of catalase or whether this is added at the end of incubation.

In order to establish the mechanism by which H_2O_2 decreases the activity at neutral pH, incubations were carried out in the presence of an excess of H_2O_2 added to the incubation mixture in the presence and absence of substrate. Fig. 4 shows that

diamine oxidase activity is not modified by H_2O_2 added to the enzyme alone, but it is immediately abolished on addition of substrate.

The results reported above suggest that diamine oxidase activity is inhibited by H_2O_2 produced during the oxidation of the substrate. Possibly, the H_2O_2 is used in the oxidation of certain groups which are important for the enzyme activity and which correspond to ionizable residues. In fact the inactivation appears only at neutral pH where H_2O_2 is lower than expected while at alkaline pH its amount corresponds to that calculated from the stoichiometry of the reaction. The absence of inactivation when H_2O_2 is added to the incubation mixture (at neutral pH) in the absence of substrate shows that the enzyme has to be in the reduced form, as observed in pea seedling diamine oxidase[3].

It is premature to make deductions about the possible functional groups affected by H_2O_2. Oxidation of copper, essential for the enzyme activity, might be excluded, because electron paramagnetic resonance results[6] show that the metal is divalent in pig kidney diamine oxidase and does not change valency during the catalytic action. The oxidation of SH functional groups seems unlikely because preliminary experiments showed that *p*-chloromercuribenzoate and *N*-ethylmaleimide, at neutral pH, have no effect on diamine oxidase activity.

A possible explanation of these results could be found in a conformational change of the protein associated with the catalytic activity; this change would unmask functional groups which are essential for catalytic activity and these would be the ones modified by H_2O_2.

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Received October 3rd, 1966