A POTASSIUM IODIDE STIMULATED PEROXIDASE FROM GOAT SUBMAXILLARY GLAND

Hatha N. Hati*, Manjusree Bal**, & Asoke G.Datta
Indian Institute of Experimental Medicine, Calcutta-32.

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Recently it has been reported from our laboratory (Hati and Datta, 1967) that the microsomal fraction of goat submaxillary gland contains a new enzyme which catalyzes the formation of diiodotyrosine (DIT) from monoiodotyrosine (MIT) in presence of inorganic iodide. It has been established beyond any doubt that thyroid preparations require hydrogen peroxide for catalyzing iodination of tyrosine (Alexander, 1959; DeGroot and Davis, 1961; Klebanoff, Yip and Kessler, 1962; Hosoya, Kondo and Ui, 1962; Igo, Mahoney and Mackler, 1964). Our enzyme also requires H_2O_2 or H_2O_2- generating system for its activity (Hati and Datta, 1967). Evidences are presented in this paper that the microsomes of goat submaxillary gland contain a new type of peroxidase which is stimulated by KI. On the other hand Yip (1966) has reported inhibition of peroxidase activity by iodide ion. As far as we know, nobody has reported a KI-stimulated peroxidase. Thus, it was thought worth while to report some of the properties of this peroxidase, which has been recorded in the present communication.

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* Junior Research Fellow of Council of Scientific and Industrial Research (CSIR).

** Senior Research Fellow of CSIR.

EXPERIMENTAL

The microsomes of goat submaxillary gland were isolated by the same procedure as described earlier (Hati and Datta, 1967) with the exception that 0.05 M phosphate buffer, pH 6.0 was used for homogenization of the gland instead of Tris buffer. The isolated microsomes were solubilized by treating them with 2% sodium desoxycholate with constant stirring for 30 minutes at 0°. The supernatant, after centrifugation for 1 hour at 105,000 x g, was used for measurement of peroxidase activity. The enzyme activity was assayed by following the optical density increase at 460 mu in a Zeiss spectrophotometer using 1 cm light path. O-dianisidine was used as hydrogen donor. The reaction mixture contained the following reagents in a final volume of 3 ml: 150 umoles of phosphate buffer, pH 6.0; 2 umoles O-dianisidine dissolved in methanol, 350 mumoles of H_2O_2; 500 mumoles of KI and 0.05 ml of the enzyme preparation containing 130 to 180 ug of protein. The enzyme was added last to start the reaction. O-dianisidine solution was prepared daily and kept in a dark bottle. Protein was determined by the method of Lowry, et al (1951).

RESULTS AND DISCUSSION

It can be seen from Fig. 1 that solubilized microsomes of goat submaxillary gland contain significant amount of peroxidase activity which is completely abolished if H_2O_2 is omitted from the reaction mixture. On boiling the preparation, the enzyme activity is completely lost. It is further interesting, to note that the enzyme loses its activity to a great extent in the absence of added potassium iodide. No change in the optical density at 460 mu has

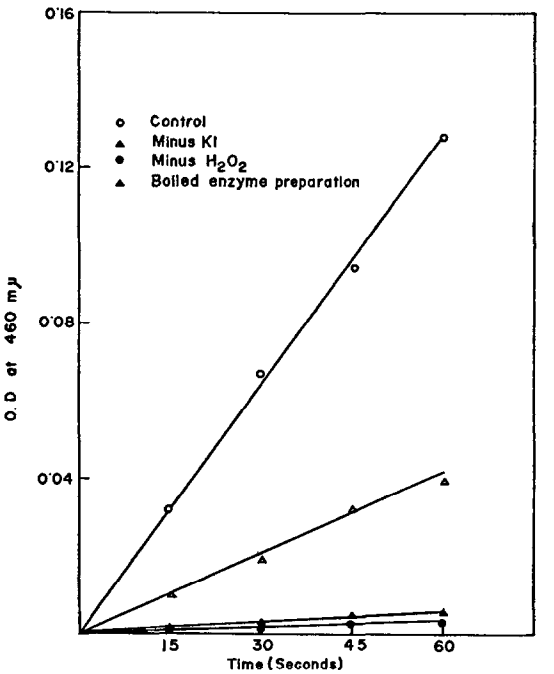

Fig. 1. Demonstration of peroxidase activity in the solubilized microsomes of goat submaxillary gland. Additions and experimental procedures have been described in the text.

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been observed in the absence of O-dianisidine. This observation indicates that the stimulation of this peroxidase activity on addition of KI is not due to having any absorbancy change at or near 460 mu caused by KI or any of its oxidized form. Rather it supports the idea that the increased optical density change in presence of KI is due to increased oxidation of O-dianisidine.

Recently, Yip (1966) has demonstrated that highly purified peroxidase from beef thyroid is inhibited by I^-. It may be seen from Fig. 2 that the increase in the enzyme activity is proportional to potassium iodide concentration. Though this proportional-

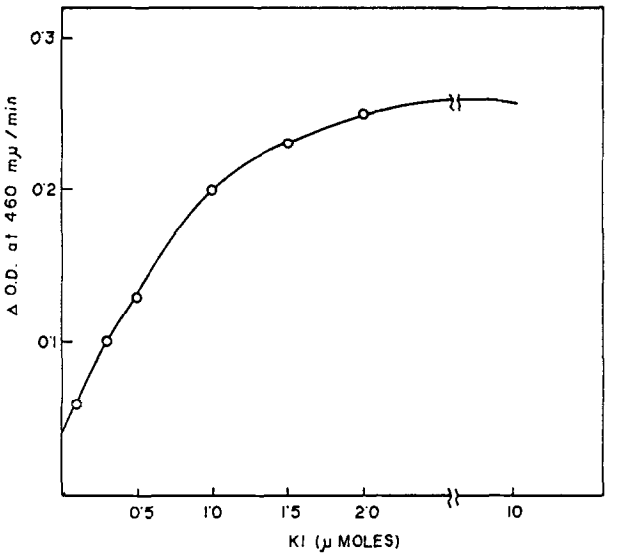

Fig. 2. **The effect of different concentrations of KI on the peroxidase activity.** KI was added in the concentrations as mentioned in the figure. Rest of the additions and procedures was same as in figure 1.

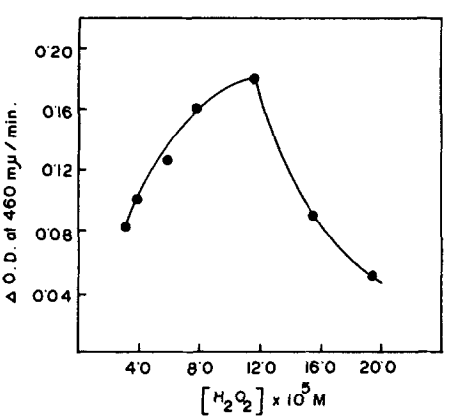

Fig. 3. **The effect of different concentrations of H_2O_2 on the peroxidase activity.** H_2O_2 was added in the concentrations as mentioned in the figure. Rest of the additions and procedures was same as in figure 1.

ity decreases with increasing concentrations of KI, no inhibitory effect is observed even at a concentration of 1×10^{-3} M.

The effect of varying concentrations of H_2O_2 on the peroxidase activity of the enzyme preparation is also interesting. It can be seen from Fig. 3 that H_2O_2 has an optimum concentration of 0.12 mM and the Lineweaver-Burk plot, obtained under these conditions, is found to be linear between 0.03 to 0.12 mM H_2O_2. Km of the enzyme for H_2O_2 is found to be 6.7×10^{-5} M.

The peroxidase activity is found to be inhibited by some potent peroxidase inhibitors like cyanide, azide, thiourea and thiouracil (Fig. 4). The enzyme has a maximum activity at pH 6.0.

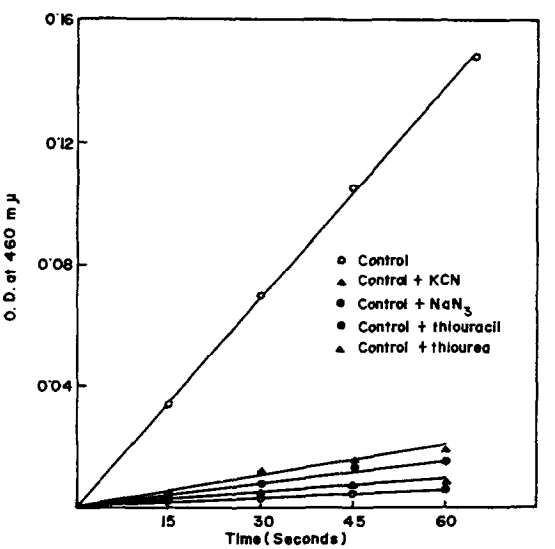

Fig. 4. The effect of some inhibitors on the peroxidase activity. All the inhibitors were added to a final inhibitor concentration of 10^{-4} M. Rest of the additions and procedures was same as described in the text.

Hence, it may be summarized that the microsomal fraction of goat submaxillary gland contains a strong peroxidase activity besides the MIT iodinating activity. It is further interesting to note that this peroxidase is stimulated by more than 300% on addition of KI. Purification of the peroxidase as well as the iodinating activity of the microsomes is in progress and only after extensive purification it will be possible for one to state definitely whether the iodinating enzyme is dependent on this peroxidase or not.

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