Effect of peroxidase on the reconstructed succinoxidase system

Little is known about the biological function of the peroxidases. We have been interested primarily in studying the properties of the uterine peroxidase and lactoperoxidase with specific reference to their abilities to function in substrate oxidation[1,2].

Spectrophotometric observations indicate that the peroxidase system can oxidize both cytochromes *b* and c_1 of the SC preparation described by CLARK *et al.*[3]. In the presence of approx. 0.2 μM lactoperoxidase and H_2O_2, reduced cytochrome *b* was oxidized rapidly while cytochrome c_1 required 2–3 min. The cytochromes were reduced with a trace of dithionite or with glucose and glucose oxidase. The latter two reagents serve to generate H_2O_2, but low concentrations of H_2O_2 added directly could be used.

In the presence of the reconstructed succinoxidase system, lactoperoxidase and the uterine peroxidase inhibit oxygen uptake. The results obtained using lactoperoxidase are shown in Table I. The succinoxidase system was reconstructed so that the concentration of the SC preparation regulated the oxygen uptake. Increased concentrations of cytochrome *c* or of the cytochrome oxidase preparation had no effect on the inhibition caused by lactoperoxidase. Increased concentrations of the SC preparation decreased the inhibition by lactoperoxidase.

TABLE I

INHIBITION OF OXYGEN UPTAKE BY THE SUCCINOXIDASE SYSTEM IN THE PRESENCE OF LACTOPEROXIDASE

Oxygen uptake was measured at 5-min intervals for 30 min at 30° using a Warburg respirometer. The control system contained 0.09 mmole succinate, 0.02 μmole cytochrome *c* (Sigma), 0.1 ml SC preparation diluted 5-fold, and 0.2 ml cytochrome oxidase preparation[4]. The total volume was adjusted to 3.0 ml with 0.1 *M* phosphate, pH 7.4. Lactoperoxidase (approximately 3 μM) was prepared according to the procedure of MORRISON *et al.*[5].

System	$\mu l\ O_2/30$ *min*	*Inhibition (%)*
Control	129	0
Control + lactoperoxidase* (0.2 ml)	55	57
Control + lactoperoxidase (0.4 ml)	24	81

* Addition of the H_2O_2 was unnecessary to observe inhibition of succinoxidase; presumably H_2O_2 was generated by the system.

In attempting to locate the site of action of peroxidase, succinic dehydrogenase and succinate-cytochrome *c* reductase activities of the SC preparation were examined. No effect of lactoperoxidase, with or without added H_2O_2, was observed on succinic dehydrogenase activity as measured by the rate of reduction of 2,3′,6-trichloroindophenol[6]. The succinate-cytochrome *c* reductase activity was inhibited by lactoperoxidase providing H_2O_2 was present. The results are shown in Table II. Under the conditions of the experiment, peroxidase and H_2O_2 did not oxidize reduced cytochrome *c*. Lactoperoxidase therefore interferes with the reduction of cytochrome *c*, presumably by oxidizing cytochromes *b* and c_1, thus accounting for inhibition of the reconstructed succinoxidase system.

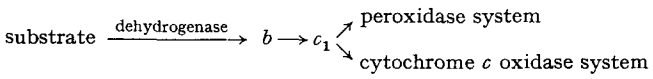
From the preliminary results reported, it would appear that a peroxidase system could function in substrate oxidation by competing with the cytochrome oxidase-cytochrome *c* system for the oxidation of cytochromes *b* and c_1, as illustrated.

TABLE II

EFFECT OF LACTOPEROXIDASE ON THE SUCCINATE-CYTOCHROME *c* REDUCTASE ACTIVITY IN THE PRESENCE OF GLUCOSE AND GLUCOSE OXIDASE

Succinate-cytochrome *c* reductase activity was determined by measuring the rate of reduction of cytochrome *c* at 550 mμ using the Beckman DU model spectrophotometer according to the procedure of CLARK *et al.*[3]. A unit of activity is defined as the Δ absorbance/min/ml SC preparation. The control system contained 0.06 mmole succinate, 4 μmoles cyanide, 0.04 μmole cytochrome *c* and 0.1 ml of the SC preparation diluted 200-fold. The total volume was adjusted to 3.0 ml with 0.1 *M* phosphate, pH 7.4. Lactoperoxidase was prepared as indicated under Table I. Glucose, 0.01 mmole, and glucose oxidase (Worthington), 0.1 ml of a 1 % solution, were added as indicated. The final volume was adjusted in each instance to 3.0 ml.

System	*Activity (units/ml)*	*Inhibition (%)*
Control	88	0
Control + lactoperoxidase (0.05 ml)	90	0
Control + glucose + glucose oxidase	91	0
Control + glucose + glucose oxidase + lactoperoxidase (0.005 ml)	44	50
Control + glucose + glucose oxidase + lactoperoxidase (0.05 ml)	0	100



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[1] F. V. LUCAS, H. A. NEUFELD, J. G. UTTERBACK, A. P. MARTIN AND E. STOTZ, *J. Biol. Chem.*, 214 (1955) 775.
[2] H. A. NEUFELD, A. N. LEVAY, F. V. LUCAS, A. P. MARTIN AND E. STOTZ, *J. Biol. Chem.*, 233 (1958) 209.
[3] H. W. CLARK, H. A. NEUFELD, C. WIDMER AND E. STOTZ, *J. Biol. Chem.*, 210 (1954) 851.
[4] L. SMITH, *J. Biol. Chem.*, 215 (1955) 833.
[5] M. MORRISON, H. HAMILTON AND E. STOTZ, *J. Biol. Chem.*, 228 (1957) 767.
[6] H. A. NEUFELD, C. R. SCOTT AND E. STOTZ, *J. Biol. Chem.*, 210 (1954) 869.

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