Sustained Oscillations in a Lactoperoxidase, NADPH and O_2 System

PERIODIC phenomena are common in biology, and the nature of the oscillator which drives them is of great interest. Recently clear oscillations at the level of enzymatic reactions have been reported in the glycolytic system[1-3]. Damped oscillations have also been found in the horse-radish peroxidase systems which oxidize reduced pyridine nucleotide[4,5] and indoleacetate or dihydroxyfumarate[6]. The peroxidase system oscillated between ferriperoxidase and compound III (possibly ferriperoxidase–O_2^- complex) in the same oscillatory cycle of oxygen consumption. The conditions which gave stable oscillations were rather limited and the oscillation ceased after several cycles even when the reaction was started from an optimal condition. Acid *p*H favoured oscillation[5].

Our recent interest has been to construct an oscillatory reaction at neutral *p*H in the peroxidase system. Lactoperoxidase was found to catalyse the oxygen-consuming oxidation of reduced pyridine nucleotides. Like horse-radish peroxidase, lactoperoxidase was converted into compound III in the presence of oxygen and reduced pyridine nucleotide. Unlike horse-radish peroxidase, however, lactoperoxidase compound III, did not rapidly decompose into ferriperoxidase when the oxygen concentration fell below a certain limit. The rapid decomposition of compound III seemed to be essential for stable oscillations in the peroxidase reaction and might be the reason why typical oscillations could not be observed in the simple lactoperoxidase system.

Methylene blue was found to promote the decomposition of compound III. Phenols such as 2,4-dichlorophenol were necessary for the peroxidase–oxidase system to maintain high activity, especially at neutral *p*H. As shown in Fig. 1, when glucose-6-phosphate dehydrogenase was added to the solution containing lactoperoxidase, 2,4-dichlorophenol, glucose-6-phosphate, NADP and oxygen, lactoperoxidase was gradually converted into compound III with concomitant uptake of oxygen. The rate of oxygen consumption became very steep when about 30 per cent of the peroxidase had been converted. In these conditions, however, the reaction reached the steady state after an overshoot in the compound III concentration, and oxidation continued rapidly, as shown by the low level of steady state concentration of the oxygen. During that time about 54 per cent of the enzyme remained in the form of compound III.

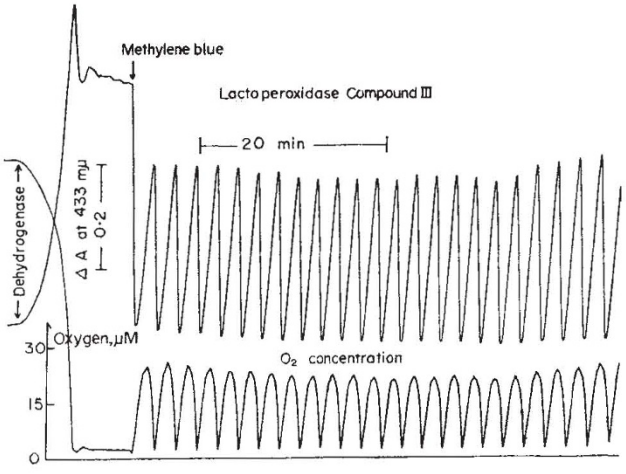

Fig. 1. Oscillatory oxidation of NADPH in the lactoperoxidase systems. The system contained 27 μM lactoperoxidase, 20 μM 2,4-dichlorophenol, 2 mM NADP, 20 mM glucose-6-phosphate and 0·1 M phosphate (*p*H 7·0). The reaction temperature was 35° C. 5·5 per cent oxygen (diluted with N_2) was bubbled into the reaction solution at a rate of 4 ml./min and the oxygen concentration in the solution was measured polarographically[5]. The reaction was started by the addition of a proper amount of glucose-6-phosphate dehydrogenase. When 6 μl. of 1 mM methylene blue (final concentration 1 μM) was added to the reaction solution, the reaction pattern changed from the steady state into the oscillatory state. The use of a high concentration of peroxidase seemed essential for the lactoperoxidase system to cause stable oscillation. Lactoperoxidase was purified from fresh cow's milk according to the method of Morrison and Hultquist[7].

Very rapid decomposition of the compound started immediately after a trace amount of methylene blue was added to the steady state. The oxygen-consuming oxidation was completely inhibited and oxygen began to accumulate in the reaction solution. The increase in oxygen concentration was followed by the conversion of lactoperoxidase into compound III. When about 30 per cent of lactoperoxidase was transformed a rapid consumption of the oxygen started again, and was followed by a decrease in oxygen concentration and the decomposition of compound III. These sequential reactions made up one oscillation cycle. The oscillations were very stable in these conditions and repeated more than fifty times before the level of reduced pyridine nucleotide became insufficient. The lowering of this level is caused mostly by a drift of redox equilibrium and it is inevitable in these conditions.

The analysis of the elementary reactions involved here has not been completed, and the detailed mechanism is now under investigation.

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Resonance Constants and the Activities of Indolealkylamines on Stomach Muscle

INDOLES in nature display an extraordinary variety of biological actions[1]. We describe here a correlation between the resonance constants of a series of indoleakylamines and their potency in causing contraction of stomach.

The potencies of 5-substituted tryptamines and 5-substituted α-methyltryptamines in causing contraction of isolated stomach strip decrease in the order: hydroxy, methoxy, chloro, methyl and hydrogen[2]. This order correlated linearly with the resonance contribution[3] of the substituents to the indole ring, biological activity increasing with decreasing resonance constant (Fig. 1). Neither partition coefficient (measured between 1-octanol and 0·1 N phosphate, *p*H 7·0) nor the pK_a of the primary amino-group correlated with activity (Table 1). The resonance constant reflects the effect of the substituent on charge distribution in the π-electron system[4] and the high negative value of the activity constant implies that high electron density at the active site may be associated