Short Communication

Transfer RNA-Peroxidase Interaction

INHIBITION OF INDOLE-3-ACETIC ACID OXIDATION

Received for publication December 4, 1979 and in revised form June 3, 1980

Tsung T. Lee
Research Institute, Agriculture Canada, University Sub Post Office, London, Ontario, Canada N6A 5B7

ABSTRACT

Transfer RNA from wheat germ, yeast, and Escherichia coli inhibited the indoleacetic acid (IAA)-induced spectral change in horseradish peroxidase (EC 1.11.1.7) and the decarboxylation of IAA. The inhibition was limited to a delay after which the increase in \( A_{427} \) and the decarboxylation of IAA resumed at the same rate as in the control; the duration of the inhibition was dependent on, but not proportional to, the concentration of tRNA. Alkaline hydrolysis destroyed the inhibitory activity of tRNA. The inhibition was completely abolished when the tRNA was added 30 seconds after IAA. Thus, the tRNA appears not to react with the enzyme intermediates formed during the reaction with IAA. The inhibition by tRNA was rapidly reversed by \( H_2O_2 \) or additional IAA, but not by 2,4-dichlorophenol. Results suggest that the tRNA interferes with the initial reaction between IAA and the heme moiety of free peroxidase, thus preventing the formation of highly active enzyme intermediates essential for IAA degradation.

During studies of binding of metabolites of IAA to yeast tRNA, the author has observed that the tRNA had an inhibitory effect on the peroxidase-catalyzed oxidation of IAA. Transfer RNAs are known to participate in other functions in addition to their primary role in ribosome-mediated protein synthesis (11). Lehman et al. (7) reported an inhibition by RNA of deoxyribonuclease activity in Escherichia coli. Jacobson (4) reported that a specific isoacceptor tRNA in Drosophila inhibited the tryptophan pyrrolase activity. Hatfield and Burns (3) reported a binding of leucyl-tRNA to an immature form of threonine deaminase, thus preventing the formation of an active form of the enzyme. Since peroxidase occurs widely in plants and many studies suggest a regulatory role of peroxidase in plant metabolism, a possible tRNA-peroxidase interaction is of interest. This paper presents data showing an inhibition of IAA oxidation by tRNA from wheat germ, yeast, and E. coli through an interaction with the heme moiety of HRP.  

MATERIALS AND METHODS

HRP (EC 1.11.1.7, Sigma type VI) with an RZ (A_{400}/A_{250}) value of 2.9 was used and changes in \( A \) upon addition of IAA were followed at 427 nm with a Unicam 8000 automatic recording spectrophotometer. The wave length 427 nm is the isosbestic point of the enzyme intermediates and was used to monitor the trans-

formation of free HRP (1, 2, 9). The composition of the reaction mixture is given in the Figures. The reaction was started by injecting 10 \( \mu l \) IAA at zero time into the cuvette under continuous stirring. The temperature was maintained at 25 \( ^\circ \)C by a thermostatically controlled cell compartment. Stock solutions of tRNA from wheat germ (type V), E. coli strain W (type XXI), and yeast (type X) (all from Sigma) were prepared with 2 \( \text{mm} \) K-phosphate (pH 6) and thoroughly dialyzed at 4 \( ^\circ \)C against the same buffer before testing. Hydrolysis of tRNA was carried out with 0.3 \( N \) KOH at 37 \( ^\circ \)C for 16 h. After hydrolysis the solution was adjusted to pH 6 and dialyzed.

Oxidation of IAA was measured by decarboxylation of \( [1^4C] \) IAA as previously described (6). The reaction mixture (2.0 ml) contained 0.1 nmol K-phosphate (pH 6), 0.2 \( \mu l \) each DCP and MnCl\(_2\), 40 nmol cold IAA, 0.68 nmol \( [1^{14}C] \) IAA (60 \( \mu Ci/\mu l \), New England Nuclear), and 7 \( \mu g \) HRP. Four or 8 nmol of tRNA were added before or after zero time. The trapped \( ^{14}C \) was counted in a Beckman model 9000 liquid scintillation system with programmed automatic quench correction.

RESULTS AND DISCUSSION

In reaction with IAA, peroxidase undergoes a series of transformations as indicated by the changes in \( A \) in the Soret region and these changes are prerequisite to decarboxylation of IAA (5, 8, 10, 12). The data presented in Figure 1 demonstrate a rapid increase in \( A_{427} \) after 40 nmol IAA was added to the enzyme

---

1 Abbreviations: HRP, horseradish peroxidase; DCP, 2,4-dichlorophenol.
solution. However, the spectral shift was completely inhibited when an equimolar concentration of wheat germ tRNA was added before IAA; the inhibition was limited to a lag period as evidenced by the data obtained with low concentrations of tRNA. After the lag, the increase in $A_{380}$ resumed at the same rate as in the control. The duration of the inhibition was dependent on, but not proportional to, the concentration of tRNA. The inhibition was reversible by adding more IAA.

Inhibition of the HRP-IAA interaction by tRNA and kinetics of the inhibition were confirmed by measurement of decarboxylation of IAA and by tRNA from different sources. Figure 2 shows that a low concentration of wheat germ tRNA present at zero time caused a 10-min delay in decarboxylation of IAA. When the concentration of the tRNA was doubled, a complete inhibition of decarboxylation was observed during the 30-min period. Similarly, tRNA from yeast and E. coli both delayed the spectral change of HRP and the decarboxylation of IAA.

Nucleotides, nucleosides, and purine and pyrimidine bases individually showed no inhibitory effect even at a molar concentration 200 times that of tRNA or IAA. Alkaline hydrolysis destroyed the inhibitory activity of the tRNA.

The inhibition by tRNA of the spectral shift in HRP was rapidly reversed by H$_2$O$_2$ added at different times during the inhibition (Fig. 3), but the inhibition was not reversible by DCP. Similar results were obtained from experiments on the decarboxylation of IAA. It has been known that H$_2$O$_2$ promotes rapid conversion of free HRP into intermediate compound I and then compound II (1, 9), whereas DCP accelerates the transitions between the intermediate compounds of HRP (2). Based on these differences and the results obtained with H$_2$O$_2$ and DCP in the reversal of the inhibited spectral shift in HRP and decarboxylation of IAA, the tRNA-induced inhibition may be interpreted as an interaction between tRNA and the free HRP, but not the enzyme intermediates formed during reaction with IAA. This suggestion was tested in other experiments in which tRNA was added at various times after the start of IAA oxidation. The inhibition was rapidly reduced when the wheat germ tRNA was added to the reaction mixture 8 s after IAA and was completely abolished when it was added 30 s after IAA (Fig. 4). The results from experiments on IAA decarboxylation were similar. It appears that the tRNA inhibited only the early step(s) of the reaction between HRP and IAA. This supports the interpretation of the reversibility of the tRNA-induced inhibition by H$_2$O$_2$, but not by DCP. The limited inhibition by low concentrations of tRNA (Fig. 1) may be interpreted on the same basis.

On the basis of the above observations, it is evident that the tRNA interferes with the initial reaction between IAA and the heme moiety of HRP, thus protecting the enzyme from forming active intermediates required for subsequent degradation of IAA. The nature of the inhibition is not well understood. Nevertheless, the tRNA-peroxidase interaction may influence not only IAA metabolism but more generally other reactions in plants involving soluble peroxidase.

**Acknowledgments**—The author is grateful to J. J. Jevnikar and G. R. Lambert for expert technical assistance.

**LITERATURE CITED**

1. CHANCE B 1949 The enzyme-substrate compounds of horseradish peroxidase and peroxides. II. Kinetics of formation and decomposition of the primary and
2. Fox LR, WK Purves, HI Nakada 1965 The role of horseradish peroxidase in indole-3-acetic acid oxidation. Biochemistry 4: 2754-2763