Activation and Inhibition of Indoleacetic Acid Oxidase Activity from Peas

Abstract. The inhibition of indoleacetic acid oxidase activity by chlorogenic acid is markedly variable. Aqueous extracts of acetone precipitates from etiolated pea epicotyls contain heat-stable substances which decrease the inhibition produced by chlorogenic acid and also have a small activating effect. Low concentrations of *p*-coumaric acid (1 μm/ml) can stimulate this action. The possible role of activators and inhibitors in the in vivo regulation of indoleacetic acid oxidase activity is discussed.

It has recently been shown that caffeic acid and chlorogenic acid are potent inhibitors of crude indoleacetic acid oxidase from peas (*1*) and pineapple (*2*). Rabin and Klein (*1*) reported that the enzyme obtained from etiolated pea epicotyls by precipitation with 40 percent acetone and extraction into *p*H 5.6 buffer is inhibited 50 percent by 0.8 μg of chlorogenic acid per milliliter. We have found that with these extracts the relation between enzymatic activity and inhibition is markedly variable.

Using the same assay conditions as Rabin and Klein (*1*), we prepared extracts which varied in their sensitivity to chlorogenic acid from complete inhibition by as little as 0.5 μg/ml to no inhibition at all by 1.5 μg/ml. Generally, it was found that re-extractions of the 40 percent acetone precipitate with *p*H 5.6 buffer yielded preparations which were more strongly inhibited by chlorogenic acid. It therefore appeared likely that the initial extracts contained a substance which counteracted the inhibitory effects of chlorogenic acid.

Table 1. Effect of boiled pea extracts on chlorogenic acid inhibition of indoleacetic acid (IAA) oxidase. The enzyme was prepared according to the method of Rabin and Klein (*1*), with the following additions: After extraction of the 40 percent acetone precipitate with the phosphocitrate buffer using 25 ml/100 gm of frozen epicotyls, the remaining precipitate was extracted with an additional amount of buffer, 25 ml/100 gm of epicotyls. The latter is designated enzyme fraction 2, the former enzyme fraction 1. The digest was also prepared according the method of Rabin and Klein (*1*), and the reaction was run for 20 minutes at 30°C. After addition of the perchloric acid–ferric chloride reagent, the mixture was filtered to remove precipitated protein. The initial concentration of indoleacetic acid was 20 μg/ml.

Chlorogenic acid added (μg/ml)	Destruction of IAA (μg/ml)	Inhibition (%)
Enzyme fraction 1		
0	3.0	
1.0	1.3	57
Enzyme fraction 2		
0	13.4	
1.0	2.1	84
Fraction 2 + boiled 1 (ratio 1:1)		
0	8.9	
1.0	5.4	39
Fraction 2 + buffer (ratio 1:1)		
0	7.7	
1.0	1.3	83

Evidence for this was obtained by adding boiled extracts with low sensitivity to chlorogenic acid to enzyme which showed strong chlorogenic acid inhibition. As shown in Table 1, the boiled extract contains material which decreases the inhibitory effect of chlorogenic acid. This observation seemed related to the findings of Gortner and Kent (*2*), who showed that pineapple indoleacetic acid oxidase is strongly activated by *p*-coumaric acid and that ferulic acid is a competitive inhibitor for this activator. We therefore tested the effect of *p*-coumaric acid on the indoleacetic acid oxidase from peas. As shown in Table 2, *p*-coumaric acid not only activated this system but also decreased the effectiveness of chlorogenic acid as an inhibitor. The variability of these extracts from peas in their chlorogenic acid inhibition can thus be explained in terms of an activator which is more rapidly extracted by the buffer than the enzyme. Those extracts that contain relatively high concentrations of activator will be less sensitive to chlorogenic acid. Although the *p*H optimum of the preparation from pineapple is much lower than that from peas, our results point to similarities between the two preparations.

The activation effects of *p*-coumaric acid are dependent on concentration. With an enzyme preparation that caused 14 percent destruction of indoleacetic acid in 20 minutes at 30°C, the addition of 1,5, 50, and 200 μg of *p*-coumaric acid per milliliter gave 53, 61, 34, and 19 percent destruction, respectively. Since dihydro-*p*-coumaric acid and dihydrochlorogenic acid are as effective as the respective parent compounds, the conjugated double bond is not required for activity. Isochlorogenic acid (*3*), neochlorogenic acid (*4*), and a caffeyl ester designated "Band 510" (*5*) are as inhibitory as chlorogenic acid, while caffeic acid is somewhat more effective.

The demonstration by Briggs, Steeves, Sussex, and Whetmore (*6*) that indoleacetic acid can be transported through intact plants which after maceration show indoleacetic acid oxidase activity led these authors to suggest that

Table 2. Effect of *p*-coumaric acid on the activity and chlorogenic acid inhibition of indoleacetic acid (IAA) oxidase. The preparation of enzyme and digestion were the same as described in Table 1.

p-Coumaric acid added (μg/ml)	Chlorogenic acid added (μg/ml)	Destruction of IAA after 20 min at 30°C (%)
0	0	37
1	0	73
0	1	0
1	1	65
1	2	0

oxidase activity may not have any physiological significance. This conclusion is strengthened by several reports (*7*) that there is no apparent cause and effect relation between the indoleacetic acid oxidase activity of extracts and the concentration of indoleacetic acid in the intact plant. It seems to us, however, that these data do not exclude the alternative hypothesis, namely, that indoleacetic acid oxidase does have a physiological role. One can postulate that in the intact tissue the activity of this enzyme is regulated by the concentration of activator and inhibitor, similar to what we and Gortner and Kent (*2*) have demonstrated in in vitro experiments. In this connection, it is possibly significant that the presence of an unidentified activator from etiolated peas (*8*) and an inhibitor from peas grown in red light (*9*) has been reported. We would like to suggest, therefore, that the physiological role of the indoleacetic acid oxidase system be still considered an open question (*10*).

ERNEST SONDHEIMER
DAVID H. GRIFFIN
Chemistry Department, State University College of Forestry, Syracuse, New York

References and Notes

1. R. S. Rabin and R. M. Klein, *Arch. Biochem. Biophys.* **70**, 11 (1957).
2. W. A. Gortner and M. J. Kent, *J. Biol. Chem.* **233**, 731 (1958).
3. H. M. Barnes, J. R. Feldman, W. V. White, *J. Am. Chem. Soc.* **72**, 4178 (1950).
4. J. W. Corse, *Nature* **172**, 771 (1953).
5. E. Sondheimer, *Arch. Biochem. Biophys.* **74**, 131 (1958).
6. W. R. Briggs, T. A. Steeves, I. N. Sussex, R. H. Whetmore, *Plant Physiol.* **30**, 148 (1955).
7. G. J. VonAbrams, *ibid.* **28**, 443 (1953); A. C. Wagenknecht and R. H. Burris, *Arch. Biochem. Biophys.* **25**, 30 (1950); W. A. Gortner and M. Kent, *J. Biol. Chem.* **204**, 593 (195→ A. W. Galston and R. S. Baker, *Am. J. Botany* **38**, 190 (1951).
8. R. E. Stutz, *Federation Proc.* **14**, 288 (1955).
9. M. Furuya and A. W. Galston, *Proc. Intern. Botan. Congr., 9th Congr.* **II**, 127 (1959).
10. This work was supported in part by a grant from the National Science Foundation.

12 October 1959