THE IMPORTANCE OF PHENOLS IN THE "ADAPTIVE" FORMATION AND ACTION OF AUXIN OXIDASE

By

A. GARAY and F. SÁGI

INSTITUTE OF PLANT BREEDING, FERTŐD

(Received November 10, 1961)

The physiological significance of auxin oxidase is due to its ability to convert oxidatively the growth hormone of plants, the indol-3-acetic acid (IAA) to an inactive substance [13, 14]. Therefore, it was expected to gain a deeper understanding of some morphogenetic and growth processes by thorough examination of this enzyme [3, 7]. However, it became evident that by investigations merely on the enzyme and the substrate the knowledge of the physiological role of the auxin oxidase cannot be approached, the different inhibiting and coferment-like stimulative phenols must be taken into consideration as well [1, 5, 10]. Recently the light-dependent formation of these phenolic coferments was proved by a number of authors [1, 4, 10], but as to their physiological effect opinions have not consolidated. Dealing with this matter the authors proposed to work out the following three problems:

1) to observe the changes of total phenol content and of the activity of IAA oxidase in the course of growth;

2) to examine the role of phenols in the adaptive formation of IAA oxidase (report on this question was already given by GALSTON and DALBERG [2] as well as by PILET [8]);

3) to perform experiments in order to find out what mechanism of the auxin oxidase regulates the auxin level in vitro and what is the role of the phenols in this process.

Material and methods

The variety „Gyulatanyai édes" of *Lupinus albus* was chosen as test plant. The treatment of the samples varied according to the objects of the experiment but in every case the seeds were germinated in a dark room at 25° C temperature and then both the too rapidly and too slowly germinating grains were sorted out. The remaining homogeneous material was potted into garden earth containing 50 per cent sand and exposed to direct sunlight, for eight hours every day. Subsequently part of the seedlings were placed into darkness for 16 hours (short-day treatment) and the rest illuminated from a distance of 1.5 m with a 100 W filament bulb also for 16 hours (long-day treatment). From the leaves of these seedlings the two youngest but fully developed ones were used to estimate the phenol level and the activity of IAA oxidase. The phenol content was measured according to the method of SWAIN and HILLIS [12], whereas auxin oxidase activity was determined in WARBURG-apparatus as described by STUTZ [11]. The dialysis of the enzyme was carried out in tapwater for 48 hours. The dialysed enzyme was activated by 2,4-dichlorophenol (DCP) [11]. In measuring enzyme activity not only the oxygen

consumed during the oxidation of IAA was measured but also the decrease of the IAA quantity controlled by paper chromatography [3].

In the adaptation test the seedlings germinated in thermostate were further held in darkness, on 25°C in nutrient solution [3]. When the seedlings attained a certain height, those of 55 to 60 mm length were selected and subjected to adaptation as follows. The plants were soaked — up to the cotyledons — into a solution containing IAA of 0.1, 1.0 and 10.0 μg/ml concentration; the pH of the solution was adjusted to 6.3 by phosphate buffer. Seedlings put into a buffer solution free of IAA served as controls. The hypocotyls of the seedlings thus treated were examined for phenol content and activity of IAA oxidase. Total phenol content is always expressed in ferulic acid equivalents.

All experiments were performed in eight replications. Within ordinary fluctuations each experiment series supplied statistically confirmed results easy to replicate; these are presented in tables and graphs.

Results

1) *Phenol level and activity of IAA oxidase*

Seedlings receiving short-day treatment did not reach the height of long-day treated plants. In the rough leaf extracts of the former after a short lag-phase the auxin oxidase acted intensively, whereas the leaf extracts of taller long-day treated plants did not decompose the IAA. However, the correlation expected between plant length and IAA decomposition did not appear if dialysed extracts were used. So it is evident that the action of auxin oxidase may be related to the rate of growth only indirectly, through substances inhibiting enzyme activity and ready for dialysis. This supposition is confirmed by the fact that in long-day treated plants — showing no IAA oxidase activity — larger amounts of phenol could be detected than in those grown under short day conditions. Results are presented in Table 1.

Table I

Length of two weeks old Lupinus albus seedlings grown under different light conditions, phenol content and auxin oxidase activity of their leaves

Treatment	Length mm	Phenol content mg per g dry weight	Auxin oxidase activity QO_2		Lag-phase of the auxin oxidase min.
			before dialysis	after dialysis	
Short-day	64.5	25.6	9.8	4.9	0—12
Long-day	110.3	32.0	0.0	4.5	∞

During the vegetation period the quantity of phenols successively increased even in plants of short-day treatment and soon attained the level inhibiting the action of IAA oxidase. Data of pertaining measurements are shown in Fig. 1, which reveals that in case of a definite phenol level the auxin oxidase

does not act in rough extracts while the activity of dialysed preparations remains unchanged.

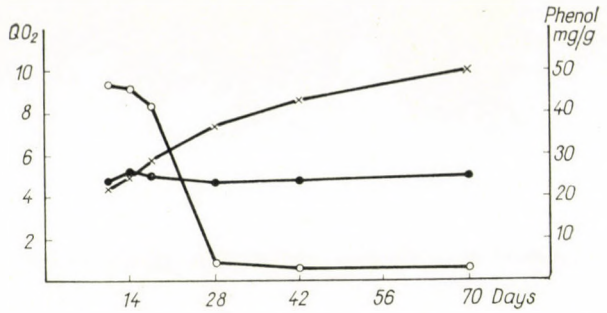

Fig. 1. Changes of phenol content and auxin oxidase activity in the leaves of short-day treated Lupinus albus seedlings during the vegetation period
Abscisse: Age of seedlings (days)
Ordinate I: IAA oxidase activity (QO_2)
Ordinate II: Phenol content expressed in ferulic acid (mg/g dry matter)
Symbols:
— × — × — = Phenol content
—○—○— = IAA oxidase activity
—●—●— = IAA oxidase activity after dialysis in the presence of DCP

2) *Apparent adaptation of IAA oxidase*

Etiolated *Lupinus* seedlings were put in IAA solution of 0.1, 1.0 and 10.0 μg/ml concentration and the adaptive increase of auxin oxidase activity was observed. Only in hypocotyls of plants put into a solution of 10.0 μg/ml concentration could a higher activity be observed. But this concentration lies

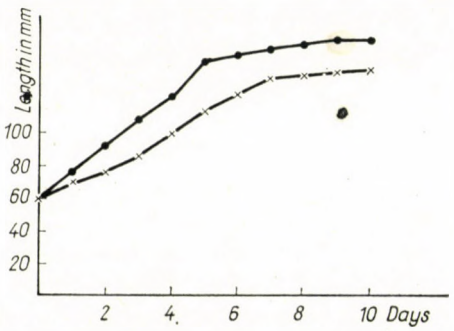

Fig. 2. Length of hypocotyls in the course of adaptive treatment
Abscisse: Number of days from the beginning of treatment
Ordinate: Length of hypocotyls (mm)
Symbols:
— × — × — = in IAA solution of 10·0 μg/ml concentration
—●—●— = in buffer solution

far beyond the physiological level, so the adaptation of auxin oxidase in *Lupinus* cannot be looked upon as typical as in peas (2). Through analysis of this phenomenon revealed that treatments with IAA of 0.1 to 1.0 μg/ml concen-

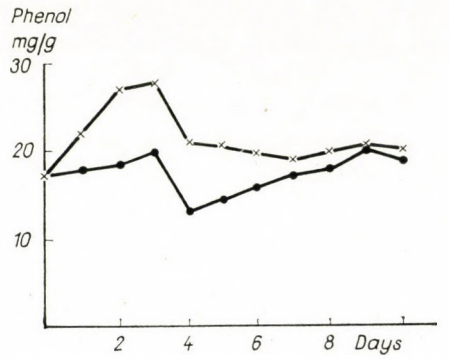

Fig. 3. Changes of phenol content during the treatment

Abscisse: Number of days from the beginning of treatment
Ordinate: Phenol content of hypocotyls expressed in ferulic acid (mg/g dry matter)
Symbols:
—×—×— = in IAA solution of 10·0 μg/ml concentration
—●—●— = in buffer solution

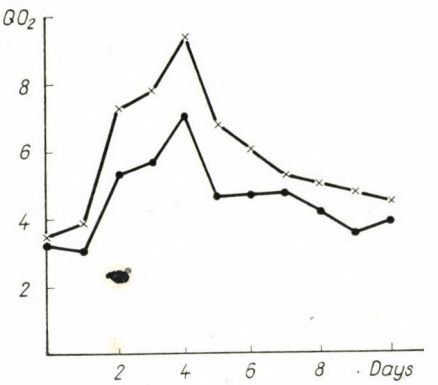

Fig. 4. Changes of IAA oxidase activity in the course of adaptive treatment

Abscisse: Number of days from the beginning of treatment
Ordinate: IAA oxidase activity (QO_2)
Symbols:
—×—×— = in IAA solution of 10·0 μg/ml concentration
—●—●— = in buffer solution

tration did not cause changes. Using IAA of 10.0 μg/ml concentration growth inhibition (Fig. 2), higher phenol level (Fig. 3) and increased auxin oxidase activity was observed. Apparently IAA oxidase was formed but the simultaneous increase of the quantity of phenols suggests caution. (Naturally, by

dialysis it could have been clarified whether a real enzyme adaptation or only an accumulation of activators took place, but seemingly in etiolated plants the enzyme is more labile, because after dialysis it could not be activated by DCP. On the other hand, the activity of non-dialysed enzyme extracted from etiolated hypocotyls was significantly stimulated by DCP.) In the present case the phenol level apparently rises thus activating the IAA oxidase. These findings are in good agreement with the results of PILET [8].

3) *The influence of DCP on IAA oxidase in vitro and the nature of the lag-phase*

What was described in the first two parts proved the dual effect of phenols on auxin oxidase, revealing, on the one hand, that a certain phenol

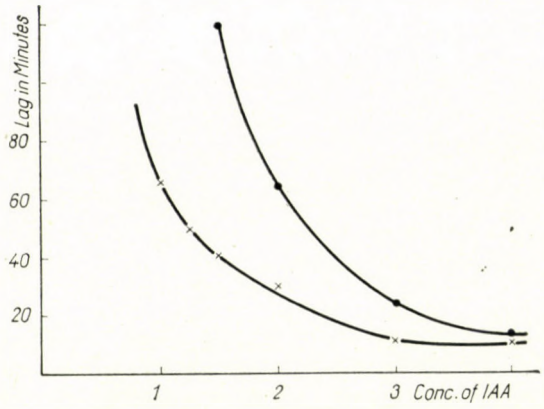

Fig. 5. Activity of IAA oxidase in vitro depending on the IAA concentration

Abscisse: Concentration of IAA as related to the quantity of homogenized leaf material
Ordinate: Lag-phase (min.)
Symbols:
— × — × — = homogenized material + IAA
— ● — ● — = homogenized material + IAA + 30 μg/ml of DCP

level inhibits the action of auxin oxidase and, on the other hand, that for the activity of the enzyme by all means a definite quantity of phenol is needed [11]. Although it is not known yet what kind of phenols are the natural indicators and inhibitors respectively in *Lupinus albus*, it seemed advisable to start in vitro experiment series in order to recognize the connection existing between the mechanism of enzyme action and phenols more intimately.

From the leaves of two weeks old *Lupinus* plants water extracts, were prepared in a proportion of 3 : 1 and incubated with different quantities of IAA and DCP in WARBURG apparatus. It seems unnecessary to report the data of all combinations, therefore only the essential results are stated showing that a diminution of IAA concentration in the reaction mixture

led to an increase of the lag-phase length. This indicates that if the enzyme is incubated with a large quantity of IAA, the oxidative decomposition immediately begins, but is preceded by a lag-phase, if the amount of IAA is small. Below a critical IAA concentration the length of the lag-phase is infinite, i. e. an extremely low quantity of IAA becomes not oxidized by auxin oxidase. In other words: for activating the auxin oxidase a certain amount of substrate is necessary. If, on the other hand, to this in vitro system phenol is added, the action of the enzyme can only be started by a larger amount of IAA. These facts are demonstrated by Fig. 5 showing also the relative concentrations of IAA. The statement of the authors is in conformity with the results of GORTNER and KENT [5] stressing that when increasing the phenol concentration the activity of auxin oxidase will remain constant in vitro only if more IAA will be added to the system.

Discussion

Knowledge of the conditions of IAA oxidase action was considerably enlarged by the investigations which clarified the importance of DCP [4], that of the interaction of Mn^{++} concentration [9]. GORTNER and KENT [5] drew the attention to the significance of the interaction of substrate level and phenol level but they neither evaluated this interference from the point of view of the physiology of auxin oxidase nor dealt with the connection of relative substrate concentration and enzyme action. It is exactly to these questions that the present study supplies new contributions, according to which the action mechanism of the auxin oxidase in *Lupinus albus* may be interpreted as follows.

The enzymatic oxidation of IAA in the plant does not start before the quantity of IAA attains a critical level and the process comes to standstill if the auxin content decreases considerably beneath the critical level. If simultaneously the endogenous phenol content is low, *(e. g.* in short-day treated plants) the auxin oxidase acts intensively and plants show a restrained growth. With increasing phenol content the auxin quantity of the tissues can reach a new critical level without beginning of decomposition. However, reaching a definite phenol level — (about 32 mg per g dry substance [expressed in ferulic acid], *e. g.* in long-day treated seedlings) — plants may augment their IAA content to a higher degree without of any enzymatic IAA oxidation, and thus provides the auxin level necessary for increased plant growth.

Of course the validity of this conception must be proved by further experiments. Investigations are also required to find out whether or not the influence of phenols and IAA on enzyme activity should be regarded as competitive [5]. But considering the lag-phase this is not probable, nor does

it influence the value of the scheme of auxin oxidase action (as described here), because by the aid of this scheme the physiological role of the enzyme may — at least in *Lupinus albus* — be better understood.

Summary

1) Phenols not only activate in vivo and in vitro the auxin oxidase but also inactivate it if a certain level is surpassed.

2) We could not demonstrate the adaptive formation of auxin oxidase, but if IAA of a physiological concentration is present the phenol level rises and activates the latent auxin oxidase.

3) To activate the auxin oxidase an adequate quantity of substrate is needed as well; so the IAA oxidase acts only if the auxin level surpasses a definite value.

4) In the presence of phenols larger amounts of auxin are required to activate the enzyme, therefore with increasing phenol level also the auxin level rises.

REFERENCES

1. Galston, A. W. (1959): Studies on indoleacetic acid oxidase inhibitor and its relation to photomorphogenesis. Photoperiodism and related phenomena in plants and animals. Washington. AAAS. 137—157.
2. Galston, A. W.—Dalberg, L. Y. (1954): The adaptive formation and physiological significance of indoleacetic acid oxidase. Amer. J. Bot. **41,** 373—380.
3. Garay, A. S.—Garay, M.—Sági, F. (1959): Photoperiodic and thermoperiodic control of indoleacetic acid oxidase in Lupinus albus L. Physiol. Plant. **12,** 799—808.
4. Goldacre, P. L.—Galston, A. W.—Weintraub, R. L. (1953): The effect of substituted phenols on the activity of the indoleacetic acid oxidase of peas. Arch. Biochem. Biophys. **43,** 358—373.
5. Gortner, W. A.—Kent, M. (1958): The coenzyme requirement and enzyme inhibitors of pineapple indoleacetic acid oxidase. J. Biol. Chem. **233,** 731—735.
6. Hillman, W. S.—Galston, A.W. (1956): Interaction of manganese and 2,4-dichlorophenol in the enzymatic destruction of indoleacetic acid. Physiol. Plant. **9,** 230—235.
7. Pilet, P.-E. (1957): Activité des auxines-oxydases et vieillissement des tissus. C. R. Acad. Sci. Paris. **244,** 371—373.
8. Pilet, P.-E. (1959): Un cas d'adaptation auxines-oxydasique (racine). Rev. gén. Bot. **66,** 45—461.
9. Reinert, J.—Schraudolf, H.—Reinert, U. (1957): Untersuchungen über Auxinoxydasen. I. Zur Frage der Identität der Enzyme verschiedener Familien höherer Pflanzen. Ztschr. f. Naturforsch. **12b,** 569—576.
10. Sági, F.—Garay, A. S. (1961): Interaction of indoleacetic acid oxidase, phenol content and gibberellic acid in the photoperiodically controlled growth of Lupinus albus L. Physiol. Plant. **14,** 488—496.
11. Stutz, R. E. (1957): The indole-3-acetic acid oxidase of Lupinus albus L. Plant Physiol. **32,** 31—39.
12. Swain, T.—Hillis, W. E. (1959): The phenolic constituents of Prunus domestica. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. **10,** 63—68.
13. Tang, Y. W.—Bonner, J. (1947): The enzymatic inactivation of indoleacetic acid. I. Some characteristics of the enzyme contained in pea seedlings. Arch. Biochem. Biophys. **13,** 11—25.
14. Wagenknecht, A. C.—Burris, R. H. (1950): Indoleacetic acid inactivating enzymes from bean roots and pea seedlings. Arch. Biochem. Biophys. **25,** 30—52.