Hill-Activity and P700 Concentration of Chloroplasts Isolated from Radish Seedlings Treated with -Indoleacetic Acid, Kinetin or Gibberellic Acid

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The Hill-activity (reduction of DCPIP or methylviologen) and the concentration of P700 were studied in chloroplasts isolated from cotyledons of radish seedlings (*Raphanus sativus* L. Saxa Treib), which had been grown with the addition of β-indoleacetic acid (IAA), kinetin, or gibberellic acid.

1) The photosynthetic activity of young chloroplasts from 3 day old *Raphanus* seedlings is very high (c. 180 μmol O_2/mol chlorophyll $\times$ h) and decreases continuously thereafter with increasing age. The steady state Hill-activity is reached after 8 to 10 days (values of 55 to 50 μmol O_2/mg chlorophyll $\times$ h).

2) Chloroplasts from plants treated with IAA or kinetin not only exhibit higher plastoquinone levels [1, 2], but also a higher P700-content and a higher Hill-activity. The promotion effect is more pronounced with kinetin (+36 to 40%) than with IAA (+12 to 17%).

3) Gibberellic acid has a different effect on composition and activity of chloroplasts. In younger seedlings the Hill-activity appears to be somewhat stimulated, without promotion effect on plastoquinone [2] or P700 concentration. After 10 days GA_3-treated plants show signs of chlorosis combined with a strong decrease in photosynthetic activity.

4) The data clearly demonstrate that the composition and activity of the photosynthetic apparatus are under phytohormone control. IAA and even better kinetin promote the light induced formation of pigment systems and electrontransport chains. GA_3 seems to block the rebuilding of the photosynthetic apparatus under steady state conditions.

Introduction

Phytohormones are known to control many development processes in plants. They also affect the development of plastids. IAA and cytokinins were found to give rise to an increased number of chloroplasts per cell in tobacco leaf discs [3]. The formation of thyllakoids and grana can be stimulated by cytokinins [4–6] and GA_3 [7]. Many authors also describe a rise in CO_2 fixation rate induced by spraying of plants with IAA [8–10], cytokinins [11, 12], or GA_3 [10, 11, 13–15]. Photophosphorylation of chloroplasts from treated plants is stimulated by cytokinins [16, 17], by GA_3 [16–18], decreased by IAA [10, 18] and GA_3 [10] or unchanged GA_3 [19].

The data available in literature are thus in part contradictionary and do not allow for a general conclusion to be drawn concerning the influence of phytohormones on the photosynthetic activity. Yet it appears that the effect of phytohormones is age dependent and does change during the development of seedlings. From this it is clear that only kinetic studies can evaluate the role of phytohormones on the formation of the photosynthetic apparatus.

The effect of phytohormones on chloroplast prenyllipid formation in *Raphanus* seedlings has carefully been studied. IAA [1] or kinetin [2] treatment promotes chlorophyll accumulation. Enhancement of chlorophyll formation induced by cytokinins has also been described in other plants [6, 17, 20, 21]. GA_3 application in turn causes a decrease in chlorophyll content after some days [14, 15, 17, 22–24], which has also been shown in *Raphanus* seedlings [2]. IAA and kinetin in *Raphanus* seedlings stimulate the accumulation of plastoquinone-9 to a much higher extent than that of chlorophyll [1, 2]. Since plastoquinone-9 is a basic functional component of the endogenous electron transport chain, it was concluded that IAA and kinetin may increase the number of photosynthetic electron transport chains and may also influence the size of the photosynthetic units [1, 2]. This should then result in a higher photosynthetic activity of chloroplasts. GA_3 on the other hand, which decreases the level of chlorophyll and even more that of plastoquinone-9 [2] is expected to

Abbreviations: DCPIP, Dichlorophenolindophenol; GA_3, Gibberellic Acid; IAA, β-indoleacetic acid; K_1, phylloquinone, vitamin K_1; P700, chlorophyll aI.

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lower photosynthetic activity on a chlorophyll basis. To test these hypotheses we have investigated the Hill-activity of isolated chloroplasts from 3 to 8 day old *Raphanus* seedlings, which were treated with IAA, kinetin and GA_3. In addition to this we have determined the concentration of P700 as a measure for the size of photosystem I.

Methods

Radish seeds (*Raphanus sativus* L. Saxa Treib) were soaked in water for 90 min and then grown on a 10% van der Crone nutrient solution [25] (22 °C, relative humidity about 60%). After a dark period of one day the seedlings were placed into the light ('Fluora' lamps, 7500 mWatt/m^2). β-indoleacetic acid, kinetin and gibberellic acid if used were included in the nutrient solution in a concentration of 5.7, 9.3 and 28.9 μM, respectively. Cotyledons of 3, 5, 8, and 10 days old seedlings were collected and the chloroplasts were isolated in a buffer containing 0.2 M saccharose, 0.05 M Tris/HCl (pH 8), 0.01 M NaCl, and 0.005 M $MgCl_2$. The Hill reaction with dichlorophenolindophenol (33 μM) was determined spectrophotometrically and that with metlhylviologen (0.2 mM) polarographically. NH_4Cl, when added as uncoupler, was applied directly before the Hill reaction measurements in a concentration of 10^{-3} M. The concentration of P700 was calculated ($\varepsilon = 6.4 \times 10^{-4}$ l $\times$ mol$^{-1} \times$ cm^{-1}) from spectra taken after chemical oxidation and reduction [27]. The chlorophyll content was determined according to Arnon [28] and used as a reference point.

Results

Raphanus seedlings grown without the addition of phytohormones exhibit after 8 days hypocotyls of up to 5 cm length. The cotyledons are fully green after 24 h exposure to continuous white light. The largest increase in cotyledon area and volume can be seen between the 2nd and the 3rd day of germination. The elongation of hypocotyls is especially pronounced between the 3rd and 4th day after germination. Treatment with IAA has little influence on the appearance of the seedlings, whereas kinetin induces shorter hypocotyls and roots as well as somewhat smaller but thicker leaves. Application of GA_3 increases the germination rate of seeds and results in slightly longer hypocotyls (up to 20%) than in the controls. There is no visible effect on cotyledon area.

Table I. Hill-activity with DCPIP and methylviologen of chloroplasts isolated from *Raphanus* cotyledons, which were grown without (control) and with the addition of phytohormones (μmol O_2/mg chlorophyll $\times$ h) (mean values of 3 isolations, standard deviation 6%).

Age of plants	DCPIP-Reduction			
	Control	+ GA_3	+ IAA	+ Kinetin
3 days	174	178	180	202
5 days	88	95	94	122
8 days	51	56	56	86
8 days + NH_4Cl	65	78	86	139
Age of plants	**Methylviologen-Reduction**			
	Control	+ GA_3	+ IAA	+ Kinetin
3 days	43	45	45	55
5 days	22	26	25	31
8 days	21	24	24	29
8 days + NH_4Cl	29	36	34	45

Chloroplasts could easily be isolated from controls and the phytohormone treated plants. The Hill-activity of *Raphanus* chloroplasts decreases with increasing age of the seedlings. This is valid for controls and the plants treated with IAA and kinetin (Table I). IAA and kinetin treated plants show higher Hill-activity rates than control plants in all cases, both for DCPIP and methylviologen (Table I). The promotion of Hill-activity is significant for kinetin (+30 to 45%) and smaller in chloroplasts of IAA treated plants (+12 to 16%).

Application of GA_3 yields slightly higher Hill-activity rates, which are similar to those in IAA treated lants. After 8 days the Hill-activity reaches a steady state value of about 51 which is maintained thereafter in controls and in plants treated with IAA and kinetin. In chloroplasts of GA_3 treated plants we found, however, a sharp drop in Hill-activity from the 8th to the 10th day (-30%), which parallels a partial breakdown of chlorophylls [2].

To exclude the possibility that the promotion of Hill-activity in chloroplasts from phytohormone treated plants is due to uncoupling of photophosphorylation, we have added the phytohormones in concentrations of up to 0.5 mM to the chloroplasts from control plants. Even after incubation times of up to 30 min we found no influence on the Hill-activity (Table II). In addition to this we have used ammonium chloride as uncoupler, which gave

Table II. Hill-activity of chloroplasts (μmol O_2/mg chlorophyll×h) from 8 d old control plants after direct addition of phytohormones to the investigation medium (incubation time 30 min; mean values of 5 determinations, standard deviation 4%).

		DCPIP	Methyl-viologen
Control		50	20
+ GA_3	(0.29 mM)	53	20
+ IAA	(0.57 mM)	49	20
+ Kinetin	(0.46 mM)	52	22

higher Hill-activity rates in all cases. The differences in Hill-activity found here between the chloroplasts of control plants and those of phytohormone treated plants were maintained even after uncoupling.

In order to obtain some information as to whether or not the phytohormones may also change the size of the photosynthetic units we have comparatively determined the P700-concentration in 8 day old *Raphanus* seedlings (Table III). The level of P700 per total chlorophyll is similarly increased as is the Hill-activity in both IAA and kinetin treated plants. Again the promotion induced by kinetin is more pronounced than that of IAA. GA_3 does not change P700-concentration in germinating *Raphanus* seedlings.

Since the phytohormones were applied to the seeds from onset of germination (via the nutrition medium), they had direct contact with the cotyledons for several days. We thus assumed that the effects, described here, are due to a direct phytohormone action within the cotyledons. In order to prove this assumption, we have applied the phytohormones in petri dishes directly to the cotyledons, which were isolated from 3 day old etiolated *Raphanus* seedlings. After 2 days of continuous illumination we found in the phytohormone-treated cotyledons with regard to the photosynthetic apparatus the same effects as seen in the whole plants. The formation *e. g.* of plastoquinone-9 was promoted by 12% (IAA) and 26% (kinetin) and that of α-tocopherol depressed by 28% (IAA) and 35% (kinetin). Furthermore the Hill-activity of isolated chloroplasts with DCPIP and methylviologen was increased by 12 to 16% (IAA) and 26 to 29% (kinetin). This and the other prenyllipid data *e. g.* changed ratios of chlorophyll a/b and xanthophylls to carotenes indicate that the phytohormones acted within the cotyledons.

Table III. Moles of chlorophyll per 1 mol of P700 in chloroplasts from 8 d old *Raphanus* seedlings grown without (control) and with addition of phytohormones (mean values from 15 determinations, standard deviation 6%).

Control	225
+ GA_3	233
+ IAA	192
+ Kinetin	161

Discussion

The Hill-activity of *Raphanus* chloroplasts decreases in all seedlings with increasing chlorophyll content and age of the plant. This has been shown for other plants too [29] and is due to the increasing amount of chlorophyll built into the antenna of the young thylakoids. Photosynthetic reaction centers are known to be formed first upon illumination [30] and the thylakoids exhibit soon fully photosynthetic function though they are not yet fully loaded with chlorophyll. This gives rise to the initial high Hill-activity rates, which are expressed on a chlorophyll basis.

When given to the germinating *Raphanus* seedlings, kinetin and to a lower degree also IAA and GA_3 result in the formation of chloroplasts with higher Hill-activity rates than those of control plants. Kulandaivelu and Gnanam [10] found a higher Hill-activity due to uncoupling of photophosphorylation, when IAA and GA_3 were given to chloroplasts of *Dolichos lab lab* L. The possibility that the phytohormones applied to the *Raphanus* plants via the nutrition medium accumulate in the young chloroplasts and may increase Hill-activity by uncoupling has, however, been excluded for *Raphanus* seedlings. The differences in photochemical activity found here between chloroplasts from controls and phytohormone treated plants were still to be seen even after uncoupling with NH_4Cl. Furthermore the phytohormones themselves had no uncoupling effects on *Raphanus* chloroplasts.

The differences in Hill-activity rates of phytohormone treated plants described here are correlated with a different chemical composition of thylakoids. Thus chloroplasts from IAA and kinetin treated *Raphanus* plants possess a higher amount of the potential electron carriers plastoquinone-9 and phylloquinone K_1 and a lower level of carotenoids and α-tocopherol than control plants (Table IV).

Table IV. Percent increase or decrease of Hill-activity, P700, plastoquinone and K_1 contents on a chlorophyll a basis in 8 d old *Raphanus* seedlings by addition of the phytohormones IAA, kinetin and GA_3 to the nutrient medium.

	+IAA [%]	+Kinetin [%]	$+GA_3$ [%]
Hill-activity with DCPIP	+10	+ 35	+10
Hill-activity with DCPIP + NH_4Cl	+32	+115	+20
Hill-activity with methyl-viologen	+12	+ 36	+14
Hill-activity with methyl-viologen + NH_4Cl	+16	+ 53	+24
P700	+17	+ 40	− 2
Phylloquinone K_1 [31]	+ 2	+ 23	*
Plastoquinone-9 [1, 2]	+52	+ 21	−22
α-Tocopherol [1, 2]	−38	− 65	− 9
Carotenoids [1, 2]	−32	− 27	−13

* Not determined.

The function of plastoquinone-9 as terminal electron acceptor of photosystem II is well established [32] The functional site of the naphthoquinone K_1 in the photosynthetic electron transport is not yet known. Its concentration corresponds to that of other components of the electron transport chain such as cytochrome f or P700 [31]. From recent work with bromene-naphthoquinones a position near photosystem II and the quencher Q is postulated for K_1 [31, 33]. The promotion of plastoquinone-9 and K_1 accumulation on a plant and on a chlorophyll basis [1, 2, 31] indicates together with the increased Hill-activity rate that under the influence of IAA and kinetin more electron transport chains are formed in *Raphanus* chloroplasts. The simultaneous augmentation of the P700 concentration indicates that the number of reaction centers may be increased too by aplication of IAA and kinetin to the developing plant. The percent increases in Hill-activity rate and P700 content are in fairly good correspondence for both IAA and kinetin treated plants.

GA_3 when given to the growing plant acts in a different way to IAA and kinetin. It may stimulate Hill-activity in the first 8 days, but there is no increase of P700-concentration and even a decrease in plastoquinone content. The considerable loss of chlorophyll and photosynthetic activity in 10 d old GA_3 treated *Raphanus* seedlings indicates that GA_3 application – in contrast to controls and IAA or kinetin treated plants – results in an early senescence. Since the photosynthetic apparatus undergoes a steady turnover and rebuilding [34, 35], this means that GA_3 does not allow the reformation of thylakoids under steady state conditions. The data reported here also indicate that GA may have stimulatory and inhibitory effects on the photosynthetic apparatus, which are time dependent.

Similar differences in chemical composition and photochemical activity of thylakoids as described here in IAA and kinetin treated plants are also found between plants grown at high light intensity [36–38] or in blue light [39–42] and plants grown at low light intensity or red light. It is of interest in this respect that the levels of auxin and cytokinins inside the plant are under light and phytochrome control [43, 44]. Furthermore there is a close relationship between blue light and the endogenous cytokinin [45, 46] an auxin [47] levels and red light and certain GA_3 effects [45].

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