Subcellular Compartmentation of Indole-3-acetic Acid in Mesophyll Cells of *Spinacia oleracea*

B. Heilmann, W. Hartung, and H. Gimmler

Lehrstuhl Botanik I, Universität Würzburg, Mittlerer Dallenbergweg 64, D-8700 Würzburg

Z. Naturforsch. **36 c**, 679–685 (1981); received April 14, 1981

Chloroplasts, Compartmental Analysis, Indole-3-acetic Acid (IAA), IAA Conjugation, Permeability

A compartmental analysis of the distribution of the plant hormone indole-3-acetic acid (IAA) was performed with mesophyll cells of spinach leaves. The results indicated that up to about 45% of the total amount of free IAA in the laminae of spinach is localized within the chloroplasts, although these organelles occupy only about 7% of the tissue volume. This distribution is due to the dissociation properties of IAA which has a pK_a of 4,7. The chloroplast envelope is easily permeable for the undissociated acid (IAAH) and less permeable for the anion (IAA^-).

The rate of uptake of IAA by chloroplasts was linearly dependent on the IAAH concentration gradient between medium and stroma. Uptake was increased by lowering the extraplastidic pH or by alkalization of the stroma during illumination. In illuminated chloroplasts, IAA was accumulated up to 4 fold compared to the surrounding medium owing to the pH gradient between the medium (pH 7.6) and the stroma (pH 8.0). The unexpectedly high plastidic IAA concentration observed in the dark (concentration ratio stroma/medium was about 2) suggested binding of IAA to chloroplast components. Due to this binding, the IAA distribution between medium and chloroplasts must not be used for calculating the stroma pH, as is possible with ABA. However, from the different distribution of IAA in light and darkness the light-induced alkalization of the chloroplast stroma can be calculated.

Introduction

The mechanism by which plants regulate the concentration of IAA have not yet been defined. In addition to modulating rates of synthesis or degradation, intracellular compartmentation might also be involved in determining hormone levels.

Albaum *et al.* (1937) have shown that the distribution of IAA between cells of *Nitella* and the surrounding medium was determined by passive transport of the undissociated IAA species at the plasmalemma and the tonoplast, and by the pH of the three spaces involved (medium, cytoplasm and vacuole of the cells) [1].

Recently, auxin uptake into plant cells has been intensively studied (for review see [2]). But up to now, little is known about the intracellular distribution of IAA. Dela Fuente and Leopold (1972) reported that there were two pools of transportable auxin in the sunflower stem, one in the cytoplasm and the other in the vacuole [3]. Their compartmental analysis was based on efflux measurements. However, the cytoplasmic location of auxin has not yet been established. As described recently, ABA distributes between isolated chloroplasts and their suspending medium in a manner dictated by the pH gradient between medium and chloroplast stroma [4]. A similar distribution might be expected for IAA, because both IAA and ABA have similar pK_a values (4.6–4.8). This paper describes experiments on the distribution of auxin within mesophyll cells of spinach leaves.

Abbreviations: ABA, abscisic acid; [^{14}C]ABA, DL-*cis,trans*-[2-^{14}C]ABA; Chl, chlorophyll; Chlpl, chloroplasts; IAA, indole-3-acetic acid; IAAH, indole-3-acetic acid (undissociated species); IAA^-, indole-3-acetic acid (anionic species); [2-^{14}C]IAA, [2-^{14}C]indole-3-acetic acid; 2-MIP, 2-methylindolo-2,3:3′,4′-pyr-6-one.

Reprint requests to B. Heilmann.

0341-0382/81/0700-0679 $ 01.00/0

Materials and Methods

Material

Spinacia oleracea was grown in a green house (winter) or outdoors (summer). *Intact "type A" chloroplasts* were isolated from freshly harvested young leaves according to the method of Jensen and Bassham [5]. The percentage of chloroplasts which had retained their envelope during isolation varied between 80 and 93% and their capacity for CO_2 assimilation between 45 and 100 µmol $CO_2 \times mg^{-1}$ Chl $\times$ h^{-1}. *Non-aqueously isolated chloroplasts* and corresponding non-aqueously leaf fractions depleted of chloroplasts were prepared according to Heber and Willenbrink [6]. The cross-contaminations of both fractions were calculated by means of their chloro-

phyll content and the activity of ATP: pyruvate phosphotransferase (EC 2.7.1.40). *Radiochemicals:* D [U-^{14}C]sorbitol, ^{3}HHO, [2-^{14}C]IAA and [^{14}C]ABA were purchased from Amersham Buchler (Braunschweig, Bundesrepublik Deutschland).

Methods

The *extraction, purification and quantitative determination of IAA* from spinach leaves tissues were based on the procedure of Mousdale *et al.* [7], which was a modification of the method devised by Knegt and Bruinsma [8]. Quantitative estimations of the considerable losses during purification of free IAA (30 to 45%) were carried out by adding [2-^{14}C]IAA (approximately 5000 cpm) as an internal standard to all samples at the beginning of the extraction. After extraction of IAA, the fluorescent IAA derivative 2-MIP was formed in acidic anhydride and 60% (w/v) perchloric acid (reaction time 10 min). Fluorescence spectra were recorded with a spectrofluorometer SPF 500 (Aminco, Silver Spring, USA) equipped with a high-intensity xenon light source, immediately after adding water in 20 fold excess to the reaction mixture. Recording of the emission spectrum required about 30 sec. The half-time of the fluorescence decay of 2-MIP in aqueous solution is 15 min. After 4 h, another fluorescence spectrum was recorded yielding unspecific fluorescence. The initial spectrum was corrected for unspecific fluorescence.

The *uptake of [2-^{14}C]IAA into intact chloroplasts* was measured by the silicone layer filtering centrifugation technique [9]. D[U-^{14}C]sorbitol served as a non-penetrating solute and tritiated water as a rapidly penetrating compound in determining osmotic spaces of chloroplasts. If necessary, continuous illumination (24 $W \times m^{-2}$ of incandescent light) was maintained during the centrifugation.

The *uptake of [^{14}C]ABA into intact spinach chloroplasts* was analyzed as described earlier [4].

Radioactivity was determined in all experiments in a liquid scintillation counter (BF 8000, Berthold, Wildbad, Bundesrepublik Deutschland). Every sample was corrected for quenching.

The *distribution of [2-^{14}C]IAA in intact leaf cells* was determined according to the following procedure: Intact spinach rosettes, grown under field conditions, were transferred to an environmental chamber (14 h 5 $W \times m^{-2}$ at 293 K and 20 h darkness at 288 K). 1.9×10^{5} Bq [2-^{14}C]IAA in small drops were applied to 3-mm cuts in the midrib of young leaves during the light period. The whole solution was taken up within about 30 min. After another 30 min incubation period the treated leaves were plunged into liquid nitrogen, while illumination was continued. The frozen material was freeze-dried and then subjected to non-aqueous fractionation [6].

IAA derivatives were hydrolyzed by treatment with alkali as described by Bandurski and Schulze [10, 11]. The quantitative estimation of the resulting IAA was performed as mentioned above.

Each experiment was carried out 3 to 6 times in duplicate.

Results and Discussion

IAA content of spinach leaves

Table I shows the content of free IAA in spinach leaves and its dependence upon growth conditions. Proper controls established that errors resulting from contaminations of the purified assays [12] did not occur. Our results were close to values reported in the literature for other plants [13, 14]. IAA concentrations were higher in physiologically younger leaves (area < 5 cm^2) which expanded faster than in older leaves (lamina area < 20 cm^2), the growth rate of which was reduced. The small and soft cotyledons of spinach which had more or less ceased to grow, contained less IAA than the following leaves. Results of measurement of free IAA in laminae of rosettes which started to bolt suggested that the IAA concentration might secondarily increase in senescent tissue [15, 16], where the level of free tryptophan, an IAA precursor, rose during proteolysis. Since the dry weight of leaves was about 10% of the fresh weight, the overall concentration of free IAA was about 10^{-6} mol $\times l^{-1}$ in young spinach leaves. Since, as will

Table I. IAA content of spinach leaves and its dependence on growth conditions. Samples were harvested in summer.

Source	IAA Content	
	[nmol IAA × g^{-1} d.w.]	[nmol IAA × mg^{-1} Chl]
Cotyledons (greenhouse)	4.5	0.33
Young leaves (greenhouse)	9.6	0.44
Young leaves (field)	6.1	0.47
Old leaves (greenhouse)	2.7	0.17
Old leaves (field, harvested from already bolted plants)	4.5	0.54

Table II. IAA concentration of aqueously isolated intact chloroplasts from spinach leaves (lamina area about 10 cm^2) grown in the field during summer. For the calculation of the endogenous IAA concentration an average osmotic volume of 30 $\mu l \times mg^{-1}$ chlorophyll was assumed [4].

pH of the isolation media	6.1/6.7
IAA content of chloroplasts [nmol × mg^{-1} chlorophyll]	0.14
Plastidic IAA concentration [mol × l^{-1}]	6.9×10^{-6}
IAA of the total amount in a leaf localized within the chloroplasts (%)	14.5 ± 5.8

be described, most of the hormone is likely to be located within the cytoplasm which occupies no more than 20% of the cellular volume, the average concentration of free auxin within the symplast of the spinach leaf tissue is approximately 5×10^{-6} mol × l^{-1} or even higher.

IAA content of spinach chloroplasts

In order to investigate IAA compartmentation within the spinach leaf cells, we determined the IAA content of chloroplasts isolated from young rosettes (2 to 3 months old) by an aqueous procedure.

Table II demonstrates that these organelles contain considerable amounts of IAA. Auxin levels in chloroplasts are even higher than ABA levels (0.4 to 4×10^{-6} mol × l^{-1}) [4]. The concentration of free IAA within the chloroplasts was comparable to that calculated for the overall level of IAA within the protoplast. The conditions during the isolation procedure influenced the concentration of free IAA in the chloroplasts. Particularly significant were the duration of the isolation, volumina of the applied media and the hydrogen ion concentration of the isolation medium (data not shown).

Recently Gimmler *et al.* (1981) reported a high permeability coefficient of the chloroplast envelope for IAAH ($P_s = 17 \times 10^{-6}$ m × s^{-1}) [17]. This value makes losses of IAA from the chloroplasts during their isolation probably. Therefore the amount of IAA in non-aqueously isolated chloroplasts was analyzed (Table III). The IAA levels of the plastidic fractions were even higher by a factor of 2 or 3 than in aqueously isolated chloroplasts preparations (compare Table II). With the non-aqueous method 35% of the total IAA was found in the chloroplasts from darkened leaves and 43% in the chloroplasts from illuminated leaves. Accordingly, the IAA levels were higher in chloroplasts from illuminated tissue than in chloroplasts from darkened leaves. Inverse relationships were observed for the non-chloroplast part of the leaf tissue which includes cytosol, mitochondria etc. and vacuoles. The IAA contents in freeze dried leaf material which subsequently was extracted by organic solvents in the course of the non-aqueous fractionation procedure were similar to, if not slightly higher than IAA concentrations in fresh leaves.

The concentration of auxin in chloroplasts of an illuminated leaf is about 2.2×10^{-5} mol × l^{-1} and 1.7×10^{-5} mol × l^{-1} in the dark. Both values are significantly higher than those obtained with aqueously isolated chloroplast preparations. This indicates a loss of IAA during the aqueous isolation. The IAA level in the cytoplasm is difficult to determine. However, from the volume ratio vacuole/cytoplasm/chloroplasts (8/1/1) and the pH difference between

Table III. Free IAA content of non-aqueously isolated chloroplasts and of the non-chloroplast part of the leaf tissue. The percent distribution of the phytohormone was calculated according to Heber and Willenbrink (1964) [6]. The osmotic volume of chloroplasts was taken to be 30 $\mu l \times mg^{-1}$ chlorophyll as measured by the silicone layer filtering centrifugation technique [4]. Note that the IAA content per g dry weight of the different fractions cannot be directly added to get the total content, because the plastidic fraction and the non-chloroplast part contribute with different portions to the overall dry weight. Values with a * are estimations.

Conditions	Fractions of the preparation	IAA Content		Intraplastidic IAA concentration [mol × l^{-1}]	IAA Distribution in leaf cells [%]
		[nmol × g^{-1} d.w.]	[nmol × mg^{-1} Chl]		
dark	chloroplast fraction	7.98	0.35	1.7×10^{-5}	35
	non-chloroplast fraction	11.09	–	1.2×10^{-5} *	65
	total preparation	10.78	–	–	100
light (24 W × m^{-2})	chloroplast fraction	14.26	0.44	2.2×10^{-5} *	43
	non-chloroplast fraction	11.65	–	1.0×10^{-5} *	57
	total preparation	11.76	–	–	100

Table IV. Relation between free and bound IAA in non-aqueously isolated chloroplasts and corresponding cytoplasmic fractions, depleted of chloroplasts, from illuminated leaves (24 W × m^{-2}) of spinach at 293 K. The leaves were harvested 1 h after application of the hormone to the nicked midribs. Ester-IAA was defined as IAA, which was liberated by hydrolysis in 1 N NaOH (1 h, 295 K); peptidyl IAA was defined as IAA set free during 3 h of hydrolysis in 7 N NaOH at 373 K.

Fraction	Readily extractable free auxin as % of the analyzed [2-^{14}C]IAA	Bound auxin as % of the analyzed [2-^{14}C]IAA	Ratio ester-IAA/ peptidyl IAA conjugates
chloroplasts	60.8	39.2	1:1.8
non-chloroplast part	83.5	16.5	1:1.1

the vacuole and the cytoplasm (1 pH unit), an IAA concentration in the cytoplasm of about 1.2×10^{-5} mol × l^{-1} in the dark and 1.0×10^{-5} mol × l^{-1} in the light can be roughly calculated. The accumulation ratio chloroplast/cytoplasm in a leaf is about 1.4 in the dark and 2.2 in the light. Our results demonstrate an accumulation of IAA within the chloroplasts, when these organelles are photosynthetically active.

Uptake of [2-^{14}C]IAA into intact chloroplasts

Fig. 1 shows rapid [2-^{14}C]IAA uptake into spinach chloroplasts. This process was so fast that its kinetics could not sufficiently be resolved at 293 K.

As demonstrated in Fig. 2 the uptake was increased in illumination chloroplasts. When these illuminated chloroplasts were darkened, previously accumulated IAA was released up to the level existing in darkened chloroplasts. The ratio ($IAA_{chlpl.}$)/(IAA_{medium}) observed when uptake was saturated was about 2 in darkness and 4 under photosynthetic conditions. Compared to ABA uptake experiments, IAA accumulation in the chloroplasts was higher by a factor of about 2 [4]. This makes IAA unsuitable for the measurement of the stroma pH, whereas ABA uptake has been shown to be useful indicator of the stroma pH [4]. If one calculates from ABA and IAA distribution between the medium and intact chloroplasts the stroma pH according to the Henderson-Hasselbalch-equation, one gets at an external pH of 7.6 a stroma pH of 7.8 with IAA, but 7.5 in the case of ABA in the dark. In the light the stroma pH is calculated to be 8.2 with IAA and 8.0 in experiments with ABA. Reasons for the unexpectedly high accumulation of IAA in chloroplasts will be discussed later.

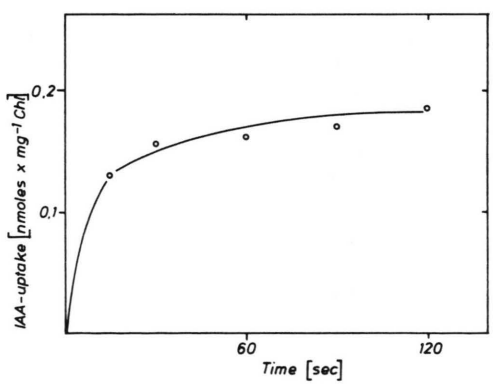

Fig. 1. Time course of IAA uptake into intact chloroplasts at 293 K in the dark. pH of the external medium: 7.6. External IAA concentration 5.7×10^{-6} mol × l^{-1}.

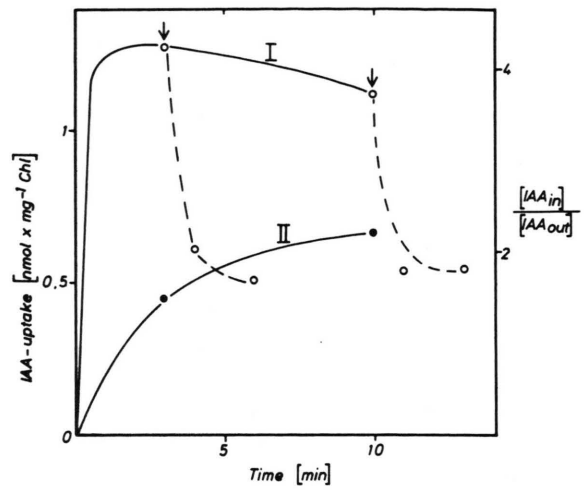

Fig. 2. The effect of light (24 W × m^{-2}) on the uptake of [2-^{14}C]IAA into intact spinach chloroplasts *in vitro*. Curve I indicates the uptake during illumination and curve II the uptake in darkness. Arrows: Illuminated samples were darkened. 293 K; pH 7.6; incubation time 5 min.

Accumulation of IAA was in the range between 10^{-7} and 2×10^{-5} mol × l^{-1} linearly dependent on the auxin concentration in the medium (Fig. 3). The IAA uptake was strongly dependent on the external H^+ concentration (Fig. 4). The influx was larger at low pH than at high pH. At pH 6.4 the ratio of intrachloroplast IAA to extraplastidic IAA concentration was as high as 11 in the light and 4 in the dark. Accordingly, the light-dark difference was

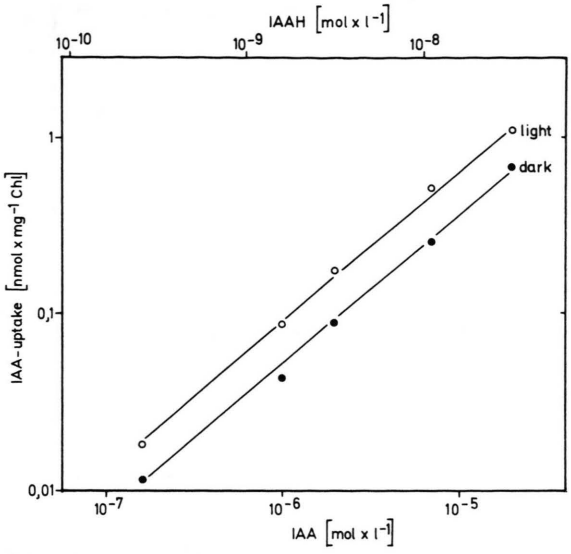

Fig. 3. Uptake of [2-^{14}C]IAA into intact chloroplasts in the light (24 W × m^{-2}) or darkness in relation to the external IAA concentration (lower abscissa-axis) and the calculated concentration of IAAH (upper abscissa-axis). 293). 293 K; pH 7.6; incubation time 5 min.

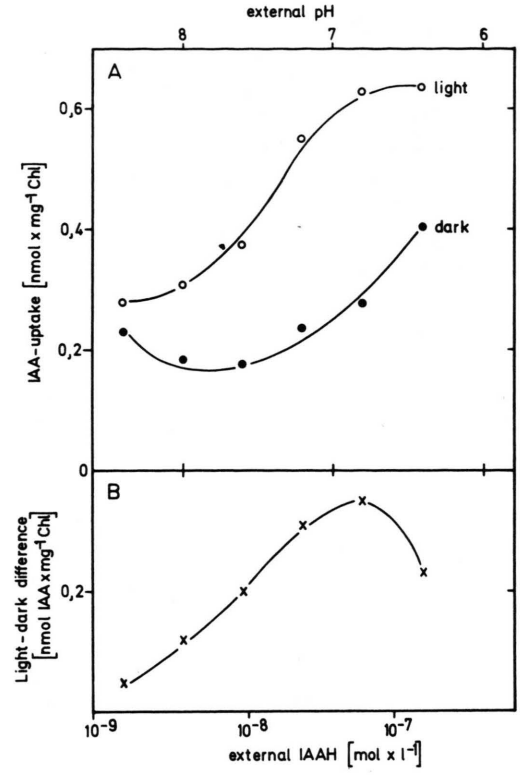

Fig. 4 A. Uptake of [2-^{14}C]IAA into intact chloroplasts depending on the pH value in the incubation medium at 293 K; external IAA concentration 6.2 × 10^{-6} mol × l^{-1}; incubation time 5 min; light 24 W × m^{-2} (○) or darkness (●). B. Difference between uptake under illumination and uptake in darkness in relation to the external IAAH concentration.

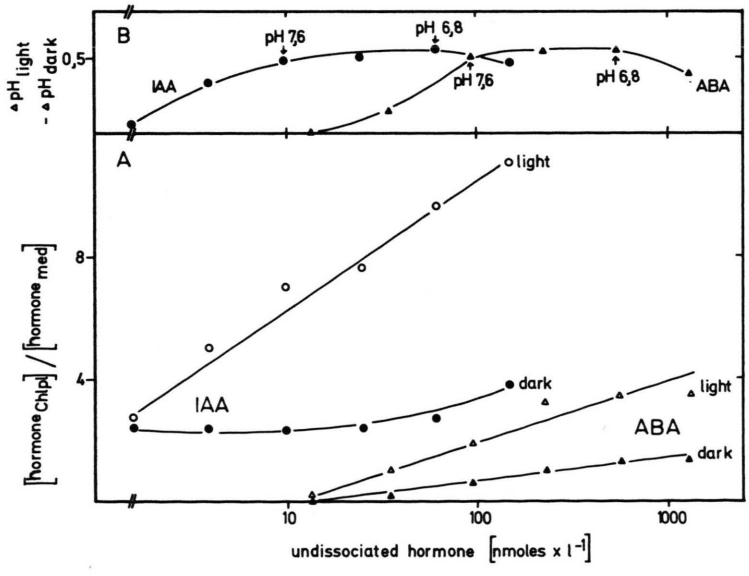

Fig. 5 A. Dependency of the accumulation ratio ($IAA_{chlpl.}$)/(IAA_{medium}) respectively ($ABA_{chlpl.}$)/(ABA_{medium}) on the external concentration of the undissociated hormone molecules in the incubation media of intact spinach chloroplasts in the dark (IAA ●; ABA ▲) and in the light (IAA ○; ABA △) at 293 K. The values of the abscissa-axis were calculated basing on the extraplastidic pH (8.4 to 6.4) and the applied phytohormone concentration: 6.2 × 10^{-6} mol IAA × l^{-1} and 5.7 × 10^{-5} mol ABA × l^{-1}. Incubation time 5 min; 24 W × m^{-2} light intensity. B. Effect of the external concentration of the undissociated phytohormone molecules (IAA ●; ABA ▲) on the difference pH existing between stroma and medium in illuminated chloroplasts minus pH between stroma and medium in non-assimilating intact chloroplasts. Conditions as mentioned under A. The pH of the plastidic osmotic space was calculated according to [4].

large at low pH values and small at high pH values. It is known that at high pH values (8.0) the H^+ concentration in the medium approaches that in the stroma of illuminated chloroplasts (Fig. 4B) [18].

In Fig. 5 IAA uptake into chloroplasts as a function of the external pH is compared with the corresponding uptake of ABA. However, different from Fig. 4, in this figure the hormone accumulation is plotted as a function of the undissociated hormone concentration, which is calculated from the external pH and pK_a. Note that the shift of ABA curves in Fig. 5 A to the right side is merely the consequence of the higher ABA concentration applied in comparison to the lower IAA concentration. Fig. 5 A demonstrates an almost linear relationship between the hormone accumulation in both light and dark and the external concentration of the undissociated hormone (compare Fig. 3). Furthermore the accumulation is always higher in the light than in the dark (compare Fig. 3). However, the light to dark accumulation ratio for both phytohormones is not constant (but compare Fig. 3). Most interesting is the fact that the accumulation of IAA is much higher than that of ABA.

Since both hormones are weak acids with comparable pK_a values and their protonated forms are assumed to penetrate the chloroplast envelope rapidly, similar stroma pH values should be calculated from the distribution of IAA and ABA between chloroplasts and medium (4, 18). Fig. 5 B demonstrates that indeed similar light-dark pH differences in the stroma can be deduced. However, Fig. 6 shows that from the IAA distribution significantly higher stroma pH values are obtained both in light and dark than in the case of the ABA distribution. Since from the latter stroma pH values similar to those found with DMO (dimethyloxazolidinedione) (data not shown) or with HCO_3^- [18] are obtained, we assume that ABA is an useful stroma pH indicator, whereas externally added IAA cannot be used to determine the stroma pH. The reason for this property could be an internal binding of IAA inside the chloroplasts.

Uptake of [2-^{14}C]IAA into intact spinach leaves, subsequent non-aqueous isolation of chloroplasts and measurement of conjugated IAA

With these experiments we investigated whether or not chloroplasts were able to conjugate applied free IAA during a relatively short time of incubation.

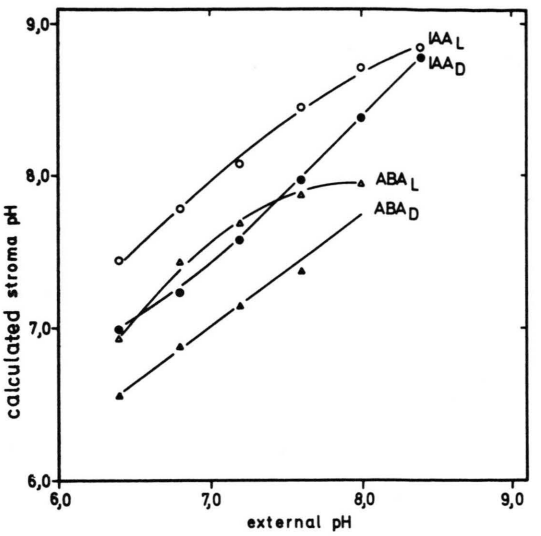

Fig. 6. Influence of the H^+ concentration in the external medium on the stroma pH of intact spinach chloroplasts under illumination (24 W × m^{-2}) or in the dark. The plastidic pH values were calculated according to the Henderson-Hasselbalch-equation by means of the accumulation ratio $(IAA_{chlpl.})/(IAA_{medium})$ respectively $(ABA_{chlpl.})/(ABA_{medium})$. External hormone concentration: 6.2×10^{-6} mol IAA × l^{-1} and 5.7×10^{-5} mol ABA × l^{-1}; incubation time 5 min at 293 K.

Leaves provide a much more convenient experimental system than aqueously isolated chloroplasts *in vitro*, because the amount of radioactive IAA incorporated into chloroplasts is too low to be analyzed for eventually formed conjugates.

Table IV demonstrates that both the chloroplast fraction and the non-chloroplast part of an intact leaf, preloaded with [2-^{14}C]IAA, contain considerable amounts of bound IAA. Corresponding experiments with [^{14}C]ABA showed that ABA was bound to a much smaller extent during the same time. Binding of IAA within the chloroplasts was significantly higher than in the cytoplasm. The chloroplast compartment contained primarily peptidyl IAA conjugates, whereas in the non-chloroplast fraction both peptidyl IAA and esterified IAA occurred at almost equal levels. The chemical characteristics indicated that the major products were 1-(indol-3-ylacetyl)-β-D-glucose and indole-3-ylacetyl-L-aspartic acid, two widespread conjugates of IAA [19].

Concluding remarks

Our results demonstrate that IAA rapidly penetrates into spinach chloroplasts. The permeating

species is assumed to be the undissociated molecule. It should be noted that our data do not suggest that the uptake of IAA into intact chloroplasts, which leads to a greater accumulation than found with ABA, is faciliated by a specific carrier, as postulated by other workers for the plasmalemma [20], but compare [17].

ABA is a convenient compound for the measurement of the stroma pH, because its conjugation or degradation [21] does not occur within chloroplasts during the used incubation periods. In contrast, chloroplasts are able to conjugate externally applied free IAA to a greater extent than the surrounding cytoplasm. These metabolised hormone molecules are therefore removed from the pool of free auxin within the chloroplasts. In consequence, further IAAH molecules can permeate the envelope by diffusion.

We observed a remarkable IAA accumulation in photosynthetically active chloroplasts, because the more alkaline compartments acted as anion traps. Both facts, dissociation and partial conjugation of intraplastidic IAA, sufficiently explain the data.

We suggest that the reactions of auxin molecules to esters and peptids are possibilities for storage and even detoxification within the homeostatic mechanism for maintenance of the intracellular IAA level. Detailed investigations to prove this assumption are in progress.

Acknowledgements

This investigation was supported by the Deutsche Forschungsgemeinschaft and by a grant of the Stiftung Stipendien-Fonds des Verbandes der Chemischen Industrie for B. Heilmann. We are much obliged to Prof. U. Heber for his courteous criticism of this manuscript.

[1] H. G. Albaum, S. Kaiser, and H. A. Nestler, Am. J. Bot. **24,** 513 (1937).
[2] M. H. M. Goldsmith, Ann. Rev. Plant Physiol. **28,** 439 (1977).
[3] R. K. Dela Fuente and A. C. Leopold, Plant Physiol. **50,** 491 (1972).
[4] B. Heilmann, W. Hartung, and H. Gimmler, Z. Pflanzenphysiol. **97,** 67 (1980).
[5] R. G. Jensen and J. A. Bassham, Proc. Natl. Acad. Sci. **56,** 1095 (1966).
[6] U. Heber and J. Willenbrink, Biochim. Biophys. Acta **82,** 313 (1964).
[7] D. M. A. Mousdale, D. N. Butcher, and R. G. Powell, Isolation of Plant Growth Substances (J. R. Hillman, ed.), p. 27, Cambridge Univ. Press, (1978).
[8] E. Knegt and J. Bruinsma, Phytochemistry **12,** 753 (1973).
[9] H. W. Heldt, Methods in Enzymology **69,** 604 (1980).
[10] R. S. Bandurski and A. Schulze, Plant Physiol. **54,** 257 (1974).
[11] R. S. Bandurski and A. Schulze, Plant Physiol. **60,** 211 (1977).
[12] D. M. A. Mousdale, J. Exp. Bot. **31,** 515 (1980).
[13] E. A. Schneider and F. Wightman, Phytohormones and Related Compounds – A Comprehensive Treatise (D. S. Letham, P. B. Goodwin, and T. J. V. Higgins, eds.), p. 29, Elsevier, Amsterdam 1978.
[14] J. R. Bearder, Encyclopedia of Plant Physiology, New Series, **Vol. 9,** Hormonal Regulation of Development I (J. MacMillan, ed.), p. 9, Springer-Verlag, Berlin 1980.
[15] A. W. Wheeler, J. Exp. Bot. **19,** 102 (1968).
[16] A. R. Sheldrake, Biol. Rev. **48,** 509 (1973).
[17] H. Gimmler, B. Heilmann, B. Demmig, and W. Hartung, Z. Naturforsch. **36 c,** 672 (1981).
[18] H. W. Heldt, K. Werdan, M. Milovancev, and G. Geller, Biochim. Biophys. Acta **314,** 224 (1973).
[19] G. Sembdner, D. Gross, H.-W. Liebisch, and G. Schneider, Encyclopedia of Plant Physiology, New Series, **Vol. 9,** Hormonal Regulation of Development I (J. MacMillan, ed.), p. 281, Springer-Verlag, Berlin 1980.
[20] P. H. Rubery and A. R. Sheldrake, Planta **118,** 101 (1974).
[21] W. Hartung, H. Gimmler, B. Heilmann, and G. Kaiser, Plant Sci. Letters **18,** 359 (1980).