Journal of Chromatography, 587 (1991) 101–108
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2372

Analysis of alanine and aspartate aminotransferase isoforms in mustard (*Sinapis alba* L.) cotyledons

Jürgen Michael Penther

Institut für Biologie II, Botanik, Schänzlestr. 1, W-7800 Freiburg i. Br. (Germany)

ABSTRACT

Two aminotransferases present in crude extracts of mustard seedling cotyledons were separated into isoforms using a Mono Q ion-exchange column. Four isoenzymes of aspartate aminotransferase were found, three of which were constitutively expressed. Only the predominant isoform exhibited changes in enzyme level that depended on plastidic factor and the nitrogen source. In comparison, alanine aminotransferase appeared to be regulated by light and the plastidic factor. After chromatographic separation, four isoforms of the enzyme were detected. The subcellular localization of these isoforms and their regulation are discussed.

INTRODUCTION

Higher plants are capable of reducing inorganic nitrate (NO_3^-), via nitrite (NO_2^-) to ammonium (NH_4^+), which is ultimately assimilated into organic substances. Enzymes required for this reduction are nitrate reductase (EC 1.6.6.1; NR) and nitrite reductase (EC 1.7.7.1; NiR). In the following assimilatory steps ammonium is first transferred to L-glutamate (Glu), an amino acid which acts as the primary acceptor molecule, and a molecule of L-glutamine (Gln) is generated. The reaction is catalysed by glutamine synthetase (EC 6.3.1.2). The following transamidation reaction between glutamine and the final acceptor 2-oxoglutaric acid (2OG), is catalysed by glutamate synthase (EC 1.4.7.1) [1]. The transfer of ammonium from glutamate to other 2-oxo acids is catalysed by different aminotransferases (EC 2.6.1.X) [2]. During the reaction of one of these enzymes the α-amino moiety of glutamate is transferred to a 2-oxo acid, *e.g.*, pyruvate or oxaloacetate [3]. The 2-oxoglutaric acid is recovered and reutilized [4]. In plants aspartate aminotransferase (EC 2.6.6.1; AsAT) and alanine aminotransferase (EC 2.6.1.2; AlAT), which are also known as glutamate oxaloacetate transaminase and glutamate pyruvate transaminase, respectively, are widely distributed. Both reactions are fully reversible. Aminotransferases are also involved in other processes, such as in the synthesis of most of other amino acids, in the hydrogen shuttle and in photorespiration [3].

At the level of aminotransferases, nitrogen metabolism, which has now become the metabolism of amino acids, diverges into several metabolic pathways.

The regulation of the levels of AsAT and AlAT in plants has not been clarified, although the temporal pattern of the appearance of these enzymes during germination has been investigated in some systems [5]. A systematic analysis of the influence of light, which acts in the mustard seedling exclusively through phytochrome (P_{FR}), has not as yet been investigated at the level of aminotransferases.

Thomas [6] reported two or three isoforms of AlAT in preparations from leaves of *Lolium tenulentum* that had been separated by DEAE-cellulose chromatography. In similar studies, Biekmann and Feierabend [7] found two isoforms of AlAT in the leaves of rye. One of these forms, which represented 90% of the total activity, was found to be located inside peroxisomes, whereas the other isoform was localized within mitochondria. In contrast, alanine synthesis from [2-^{14}C]pyruvate has been demonstrated in purified chloroplasts [8]. Other studies

0021-9673/91/$03.50

have led to the conclusion that mitochondrial and soluble isoforms are actually the same protein [9]. Collectively, these results suggest that appropriate aminotransferases are widely distributed within plant cells [8].

The separation of AsAT isoforms is much easier: after gel electrophoresis coloured bands, representing isoforms, could be detected after staining [10,11]. By this technique up to five isoforms were observed [4]. The methods used allowed the determination of the number of isoenzymes present, but not their determination. AsAT isoforms can be found in different compartments, *e.g.*, cytosol, mitochondria, microbodies and plastids [4,5].

In *Sinapis alba* L. cotyledons, AsAT enzyme activity reaches a maximum at 60 h after sowing. Only small changes in enzyme activity are observed to result from exposure to light or alteration of the nitrogen content of the growth medium (data not shown). In contrast, the AlAT level is regulated and exhibits a strong light-mediated rise. A knowledge of AsAT and AlAT levels during seedling development is necessary in order that seedlings of an appropriate age can be analysed for their isoenzyme patterns.

This paper reports the separation of AlAT and AsAT isoforms from crude plant extracts using a Mono Q anion-exchange column.

EXPERIMENTAL

Growth conditions

Seeds of white mustard (*Sinapis alba* L., harvest 1986) were produced by a commercial grower from our original seed stock [12]. They were selected and grown at 25 ± 0.5°C with 3.5 ml of water or 15 m*M* solutions of KNO_3 and NH_4Cl on four layers of paper as described previously [13]. Where accumulation of coloured carotenoids should be prevented, the herbicide Norflurazon (HF, SAN 9789) was added to the medium from sowing onwards [14].

Light treatment

Standardized light fields were used as described by Mohr and Drumm-Herrel [15]: red light (R, 6.8 W m^{-2}, $\varphi_R = 0.8$) and far-red light (FR, 3.5 W m^{-2}, $\varphi_{FR} = 0.03$). P_{FR} is the physiologically active species of the phytochrome system. A gauge for the state of the phytochrome system is given by the φ value, the wavelength-dependent photoequilibrium of the phytochrome system, which is defined as follows: $\varphi = P_{FR}/P_{tot}$ = far-red absorbing form of phytochrome/total phytochrome.

Extraction conditions

For the extraction of AlAT, twenty pairs of cotyledons were homogenized on ice with a mortar and pestle in 2 ml of extraction buffer [100 m*M* tris(hydroxymethyl)aminomethane (Tris)–HCl (pH 7.1)–6 m*M* EDTA–5 m*M* $MgCl_2$–10 m*M* DTE–63 μM pyridoxal-5-phosphate (PLP)]. Homogenization was performed in the presence of 1 g of quartz sand and 0.1 g of Dowex 1-X2 to remove phenolics and polyphenolics [16]. When Triton X-100, bovine serum albumin (BSA) (purchased from Boehringer, Mannheim, Germany; fraction V, lyophilizate, protein content >95%) or betaine was added to the extraction buffer, they were used at the following concentrations: 1.5% (v/v) Triton X-100, 0.4% (w/v) BSA and 0.4, 1.0 and 7.5% (w/v) betaine. After addition of another 4 ml of extraction buffer, the homogenate was centrifuged in the cold for 20 min at 39 000 *g*. For sonication experiments, homogenates standing on ice were sonicated three times for 15 s with ultrasonic waves of 20-kHz frequency and a power of 30 W using a Branson (Danbury, CT, USA) Model B 15 cell disruptor connected with the standard microtip, and finally centrifuged. Supernatants contained 3.5–8 mg ml^{-1} of protein and were used as crude extracts.

AsAT (EC 2.6.1.1) extractions were performed as above, except that the extraction buffer was 100 m*M* Tris–HCl (pH 7.8) and 0.15 g of Dowex 1-X2 was added prior to each homogenization.

Enzyme assay

AlAT activity was measured spectrophotometrically at 25°C by the decrease in NADH in a coupled assay using lactate dehydrogenase (EC 1.1.1.27) (LDH) as auxiliary enzyme by a modification of the method of Hørder and Rej [17]. All solutions were prepared in 100 m*M* Tris–HCl buffer (pH 7.8). The final concentrations were 100 m*M* L-alanine (Ala), 16.7 m*M* 2OG, 0.27 m*M* NADH, 0.11 μM PLP and 239 nkat of LDH.

AsAT activity was measured according to Rej and Hørder [18], but L-aspartate (Asp) was substituted for Ala and malate dehydrogenase (EC 1.1.1.37) (MDH) for LDH. The final concentrations were

67 m*M* Asp, 10 m*M* 2OG, 0.27 m*M* NADH, 0.11 μM PLP and 160 nkat of MDH.

Ion-exchange chromatography

Separation of AlAT isoforms. Extracts were either prepared as described above with detergent or by sonicating. Low-molecular-weight substances were removed and buffers exchanged by gel filtration on Sephadex G-25 according to Neal and Florini [19] followed by filtration (Millipore, Type GVWP, pore size 0.22 μm). Separations were performed with a Mono Q HR 5/5 anion-exchange column attached to a fast protein liquid chromatography (FPLC) system (Pharmacia-LKB, Uppsala, Sweden). A 4-ml volume of extract, diluted with buffer A [20 m*M* Tris–HCl (pH 8.0) containing 5 m*M* DTE] to 10 ml, was loaded onto the column. The column was equilibrated with 30 ml of buffer A and pre-eluted with 22.5 ml of buffer A. The different peaks of AlAT activity were eluted with a linear gradient of 0–100% buffer B (= buffer A plus 0.4 *M* NaCl), in a volume of 20 ml. Fractions of 0.5 ml were collected at a flow-rate of 1 ml min^{-1}.

In order to minimize aggregation effects, the zwitterion betaine was used in some experiments as described in the figure legends.

Separation of AsAT isoforms. To separate the AsAT isoforms, the Mono Q HR 5/5 column was equilibrated with 20 ml of buffer A [20 m*M* Tris–HCl (pH 8.5)]. A 1.5-ml sample containing up to 2.5 mg of protein was diluted with buffer A to 10 ml. The column was pre-eluted with 22.5 ml of buffer A. The different peaks of AsAT activity were eluted with a linear gradient of 0–40% buffer B (= buffer A plus 1 *M* NaCl) in a volume of 20 ml, followed by a step to 100% buffer B. Fractions of 0.5 ml were collected at a flow-rate of 1 ml min^{-1}. Into each of the first thirty test-tubes 0.5 ml of a linear but opposite salt gradient from 30 to 0% B was filled. This leads to an identical salt concentration of 300 m*M* NaCl in the samples.

Recovery of enzyme activity compared with the total activity applied to the column was >95% activity for AlAT and >98% for AsAT. All steps were carried out at 4°C, except some ion-exchange experiments, which were prepared at room temperature (see figure legends). All buffers were prepared at 25°C.

RESULTS AND DISCUSSION

The time course of AsAT activity reaches its maximum 2.5 days after sowing. The activity then declines and after an additional 24 h reaches a steady state. There is only a slight difference between enzyme activities of dark- and light-grown seedlings, and induction by NO_3^-, which was previously found to induce a number of nitrate assimilatory enzymes [20–22], does not occur. Rather, the level of AsAT is lower if NO_3^- is applied to seeds. The influence of NH_4^+ is also very small, although it increases the enzyme level (unpublished data). It is concluded that the appearance of AsAT is constitutive. Light, which acts via phytochrome, or different nitrogen forms (NO_3^- or NH_4^+) can only slightly alter the time course. Hence the possibility that different isoforms of the enzyme exist was investigated. After separation on an anion-exchange column, four peaks of AsAT activity were detected (Fig. 1). The first isoform, called $AsAT_1$, eluted at an approximate salt concentration of 75 m*M*. Isoform $AsAT_2$ eluted at 145 m*M*, $AsAT_3$ at 195 m*M* and $AsAT_4$ at 235 m*M*. Isoforms 1 and 3 were the most prominent enzyme components. Polyacrylamide gel electrophoresis [11] of the crude extracts demonstrated the presence of five bands of AsAT activity: three strong bands, a small band and a very small band (data not shown).

In the case of AsAT the yield of activity recovered was >120% of that activity applied to the column. This is probably the result of the influence of salt on enzyme activity. Therefore, a gradient from 0.3 *M* NaCl to buffer A (volume 15 ml, flow-rate 1 ml min^{-1}) was collected in the fraction vials before the chromatographic separation was initiated. Consequently, the salt concentration was 0.3 *M* NaCl in all fractions containing AsAT activity and the recovery was about 98%.

The elution profile of AsAT showed distinct differences only for isoform 3 (Fig. 1). $AsAT_3$ was decreased by NO_3^- but increased by NH_4^+. In herbicide-treated plants grown under continuous red light, cotyledons did not contain normal chloroplasts and only small chlorophyll-free rudiments were present. Protein, especially enzymes, and mRNAs normally contained inside the chloroplast are no longer detectable under these treatment conditions [14,23]. If the extractable enzyme level is

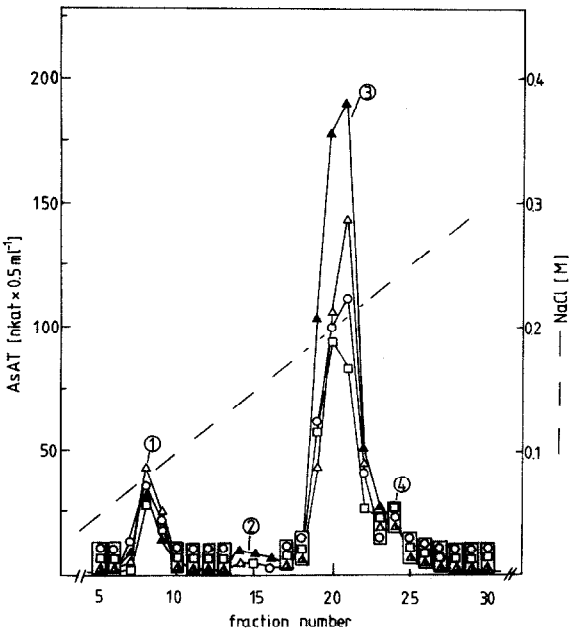

Fig. 1. Mono Q (HR 5/5) chromatography of AsAT isoforms from crude extracts of *Sinapis alba* L. cotyledons prepared with 100 m*M* Tris–HCl (pH 7.8). Extracts were centrifuged through Sephadex G-25 to change buffers. A 2.5-mg amount of protein diluted to a final volume of 10 ml with buffer A, corresponding to 1.5 ml of crude extract, was applied to the column using the Superloop. Starting buffer (A): 20 m*M* Tris–HCl (pH 8.5); gradient, 0–0.4 *M* NaCl in a volume of 20 ml, followed by a step to 1.0 *M* NaCl in a volume of 3 ml; flow-rate, 1 ml min^{-1}. No AsAT activity was detected beyond fraction 30. The data for AsAT chromatography are based on five independent experiments each. Plants were grown on (○) water, (□) 15 m*M* KNO_3, (△) 15 m*M* NH_4Cl or (▲) 15 m*M* NH_4Cl in the presence of Norflurazon ($1 \cdot 10^{-5}$ *M*) for 60 h in continuous red light (cR).

not affected by this treatment, the localization of AsAT inside chloroplasts can be excluded. Growth of plants in the presence of NH_4^+ resulted in a substantial increase in $AsAT_3$, while other isoforms are not affected (Fig. 1).

The second aminotransferase which is widely distributed among plants is AlAT. Whereas the level of AlAT in dark-grown seedling increases only slightly, a strong increase is seen in light-grown seedlings. Far-red light (cFR) is more effective than cR (unpublished data). Hence AlAT activity is regulated by light, which acts via the high irradiance reaction of the phytochrome system.

When plants are grown under photooxidative conditions (NF and cR), the AlAT level reaches a level between cD and cR (unpublished data), which indicates that the presence of intact plastids, which are capable of sending off the plastidic signal [23–25], is essential to give the full expression of AlAT activity. These data suggest a localization of an AlAT isoform inside the plastidic compartment or inside a compartment which is related to the presence of intact plastids [23].

To test this, a further experiment was necessary. Plants grown in far-red light show no effect of photooxidation. If such plants are transferred to photooxidative red light, the plastidic compartment is quickly destroyed, including most of the contained compounds (proteins, mRNAs), whereas DNA is not. Far-red light shows no effect on the AlAT level in the presence of NF (unpublished data), but the level of AlAT is not negatively affected in plants transferred to red light. Hence a plastidic localization can be excluded. These findings are in accordance with former findings that intact plastids are necessary for the appearance of peroxisomal enzymes [23] and the localization of an AlAT isoform [7,26]. Under conditions where intact chloroplasts could not develop, peroxisomal isoforms of AlAT do not appear and the enzyme level is reduced. AlAT is not affected, however, if already developed chloroplasts are photooxidized. Therefore, a localization inside peroxisomes is suggested. Surprisingly, the AlAT level increases above the normal physiological range, a phenomenon termed "superinduction". These results are not easy to explain, and an analysis of the isoform pattern was needed.

AlAT activity is not fully soluble and a detergent is often used for complete extraction. Extraction using the non-ionic detergent Triton X-100, which was optimized for the measurement of the AlAT level in crude extracts [27], leads to severe problems with protein separation, owing to lipid contamination of the column. The application of the detergent can be substituted by sonicating the homogenates before centrifugation.

If extracts were prepared by sonication rather than detergent treatment to disrupt compartments such as microbodies, the same AlAT activity was measured in crude extracts. To minimize aggregation effects between proteins themselves and other compounds of the crude extract, betaine was added to all buffers at a concentration of 1.0% or 7.5% (w/v). The result of anion-exchange chromatogra-

phy using a Mono Q (HR 5/5) column is shown in Fig. 2. With 1% betaine broadened peaks eluted over the whole gradient; 7.5% betaine did not lead to a better solution.

The separation of crude extracts prepared with detergent (Fig. 3a) but without addition of zwitterions showed nearly the same result. The major peak of AlAT activity eluted within a volume of 5 ml, a quarter of the gradient volume. Under both experimental conditions (Figs. 2 and 3a) the distribution of AlAT was very similar with one predominant "peak", followed by two smaller peaks.

When the extraction method was optimized for crude extracts, neither the use of common protease inhibitors, such as phenylmethylsulphonyl fluoride (PMSF) or leupeptin [28], nor the addition of BSA influenced the level of detectable AlAT activity. BSA only affected the stability of the extracts. Therefore, the addition of protein was chosen to protect AlAT from proteolytic digestion. Where separation into isoforms was necessary for a better

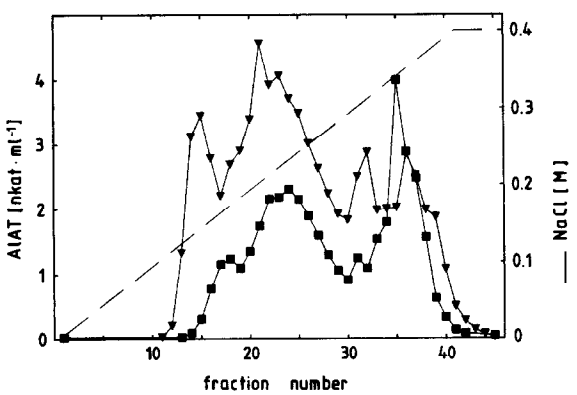

Fig. 2. Anion-exchange chromatography of AlAT isoforms present in crude extracts of cotyledons of *Sinapis alba* L. on a Mono Q (HR 5/5) column. In order to minimize aggregation effects, betaine was added to all buffers at concentrations of (■) 1 and (▼) 7.5% (w/v). Extracts were sonicated (for details, see Experimental). After gel filtration through Sephadex G-25, 4 ml of extract (3.5 mg ml^{-1} of protein) were diluted with buffer A to a final volume of 10 ml and were loaded onto the column. After elution of non-specific bindings with 22.5 ml of buffer A [20 m*M* Tris–HCl (pH 8.0)–5 m*M* DTE], the AlAT activity was eluted with a linear gradient of 20 ml rising to 0.4 *M* NaCl. The flow-rate was 1 ml min^{-1}. The data for AlAT chromatography are based on two independent experiments. Plants were grown (■) on water for 79 h in continuous red light (cR) or (▼) on 15 m*M* KNO_3 in the presence of Norflurazon ($1 \cdot 10^{-5}$ *M*) for 96 h in continuous far-red light (cFR).

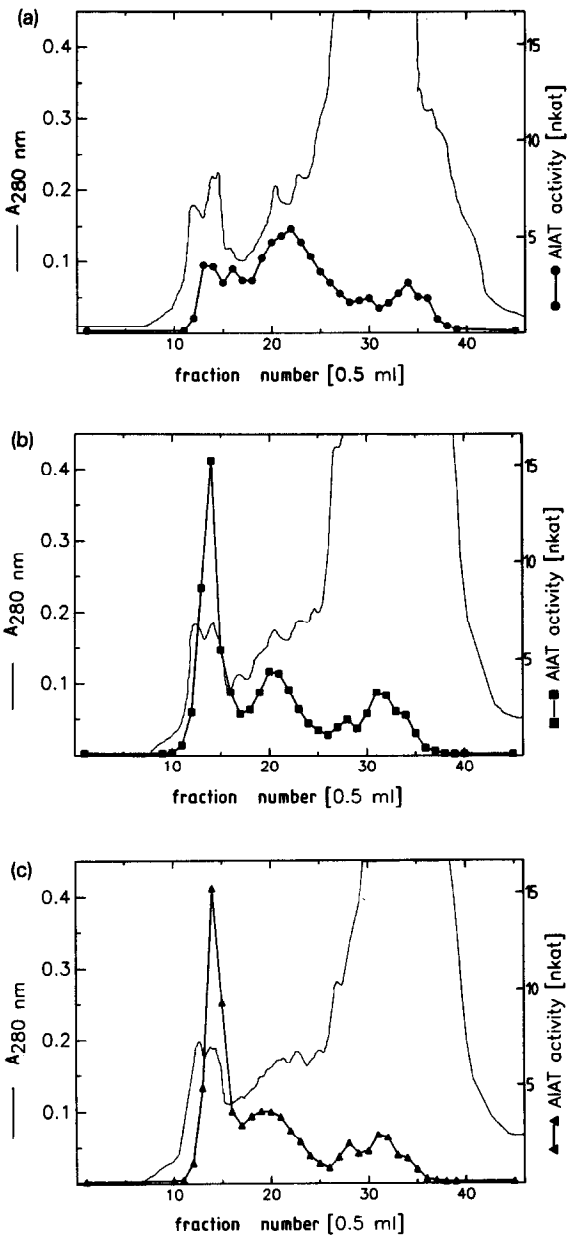

Fig. 3. Elution profile of AlAT activity present in extracts of mustard seedling cotyledons after anion-exchange chromatography on a Mono Q (HR 5/5) column. Extracts were prepared with extraction buffer containing (a) 1.5% Triton X-100, (b) 1.5% Triton X-100 plus 0.4% BSA or (c) 0.4% BSA plus sonication of the extract (for details, see Experimental). Chromatographic conditions as in Fig. 2. The data for AlAT chromatography are based on two independent experiments each. Seedlings were grown on 15 m*M* KNO_3 in the presence of Norflurazon ($1 \cdot 10^{-5}$ *M*) for 4 days in continuous far-red light (cFR).

understanding of AlAT regulation, the BSA concentration was halved, from 0.8 to 0.4%, to avoid high protein concentrations during the chromatographic step. The binding capacity of the column was not exceeded and no AlAT activity could be detected in the pre-elution volume.

The presence of a protective protein in the extraction medium caused the pattern of AlAT activity to change (Fig. 3a and b). Both extraction methods lead to the same distribution of AlAT (Fig. 3b and c): four peaks of enzyme activity were detected, a major peak followed by three minor peaks. Whereas the AlAT distribution changed significantly after protein addition, the protein absorbance at 280 nm did not (Fig. 3a and c). This indicates that chromatographic resolution is not negatively affected by addition of BSA. AlAT seems to be particularly sensitive to proteolytic digestion, which could be prevented in this way.

In order to exclude the possibility that BSA might affect non-specific binding or aggregation, the separation was repeated in the presence of 0.4% betaine (Fig. 4). As shown above (Fig. 2), higher concentrations of the zwitterionic compound betaine did not improve the resolution, so only a small amount was used for this experiment. The major peak eluted was closely followed by three peaks of enzyme activity. Differences between the curves are caused by different ages of plant material.

Both extraction methods (Figs. 3b and c and 4) lead to four peaks of AlAT activity with nearly the same position and the same distribution of activity. As the extraction procedure in the presence of a detergent leads to problems with dissolved lipids, the sonication procedure without betaine addition was chosen for extract preparation. In this instance more than 95% of AlAT activity, as compared with the total activity applied to the column, was recovered. The isoforms of AlAT (Fig. 5) are named $AlAT_1$ for the first-eluting form, at *ca.* 135 m*M* NaCl, $AlAT_2$ at 195 m*M*, $AlAT_3$ at 275 m*M* and $AlAT_4$ at 305 m*M*.

To study the regulation of the enzyme, plants were grown under different conditions. In dark-grown seedlings $AlAT_4$ is the main component and $AlAT_{1-3}$ are hardly detectable. Plants grown in the light (cR) show a dramatic increase in $AlAT_1$, a less dramatic increase in $AlAT_{2+3}$ and only a slightly increase in $AlAT_4$. Under photooxidative conditions (NF and cR) the pattern of distribution is completely different. $AlAT_{1+2}$ show levels comparable to dark-grown plants. $AlAT_3$ increases strongly and $AlAT_4$ doubles its activity compared with dark conditions. As indicated above, a plastidic localization was suggested by the results obtained with crude extracts from continuous light (cR + NF), but the "superinduction" experiment (where plants were transferred from cFR to cR) leads to different results. $AlAT_{1+2}$ shows an increase compared with plants grown in continuous red light, but this increase is normal, if compared with far-red-grown plants (data not shown). This shows that the expression of the affected isoforms is under the control of phytochrome and depends on the presence of the plastidic factor during the first 3 days of seedling development. $AlAT_4$ showed an increase that is slightly more than the detectable far-red level. Only $AlAT_3$ rises distinctly and reaches nearly the same extent as in the former experiment.

It appears that if plastidic factor is lacking, the level of $AlAT_3$ increases. This supports a recent finding, first described for the isoform 1 of NiR by Schuster and Mohr [29], at the level of mRNA

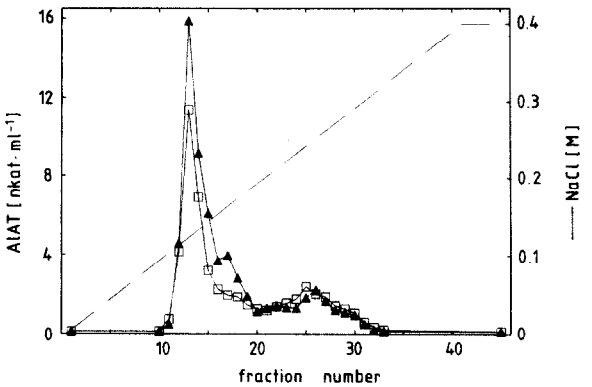

Fig. 4. Mono Q (HR 5/5) chromatography of AlAT from cotyledons of mustard. Extracts were prepared with extraction buffer containing 1.5% Triton X-100 and 0.4% (w/v) BSA. Where betaine was used (closed symbol) to minimize aggregation effects, it was present in all buffers in a concentration of 0.4%. Extracts contained up to 8 mg ml^{-1} of protein; 4 ml of extract were used in each experiment. Chromatographic conditions as in Fig. 2, except that separations were carried out at room temperature. Pre-elution showed no AlAT activity. The data for AlAT chromatography are based on two independent experiments each. The plants were grown on 15 m*M* KNO_3 for either (□) 72 h or (▲) 77 h in continuous far-red light (cFR).

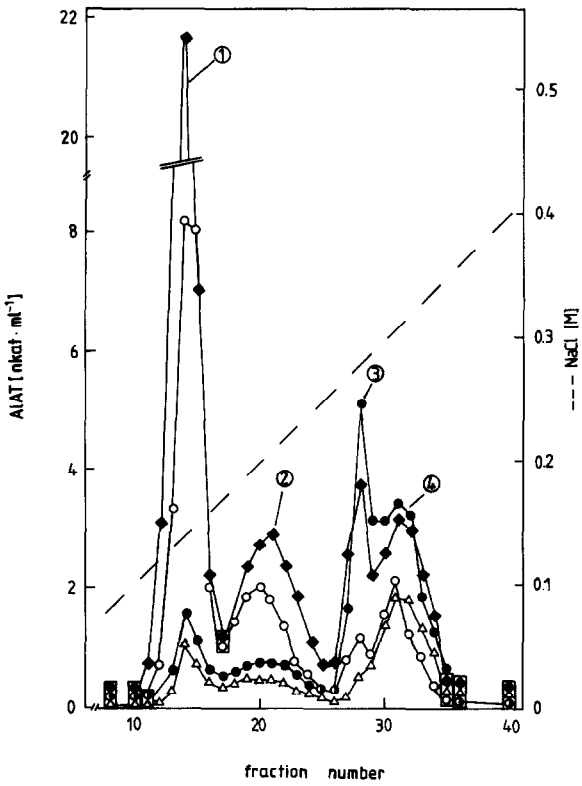

Fig. 5. Separation of AlAT isoforms from cotyledons of *Sinapis alba* L. carried out by anion-exchange chromatography (FPLC, Mono Q HR 5/5). The extraction buffer contained 0.4% (w/v) of BSA. After homogenization the extracts were sonicated (for details, see Experimental). Up to 20 mg of protein, diluted in buffer A to a final volume of 10 ml, corresponding to 4 ml of crude extract, were applied to the column using the Superloop. Starting buffer (A): 20 m*M* Tris–HCl (pH 8.0)–5 m*M* DTE; gradient, 0–0.4 *M* NaCl in a volume of 20 ml; flow-rate, 1 ml min^{-1}. Four peaks of activity were eluted ($AlAT_1$ at *ca.* 135 m*M*, $AlAT_2$ at 195 m*M*, $AlAT_3$ at 275 m*M* and $AlAT_4$ at 305 m*M* NaCl). No AlAT activity was detected below fraction 10 or beyond fraction 36. The data for AlAT chromatography are based on 3–10 independent experiments each. Seedlings were grown on 15 m*M* KNO_3 for 4 days in (△) continuous darkness (cD) or (○) continuous red light (cR) or (●) on 15 m*M* KNO_3 for 4 days in the presence of Norflurazon ($1 \cdot 10^{-5}$ *M*) under photooxidative conditions in continuous red light (cR). In order to investigate "superinduction" effects, the seedlings were grown in FR from sowing onwards for 3 days and then transferred to photooxidative R (◆).

translatable *in vitro*. It is concluded that lack of the plastidic factor can lead to an increase in proteins which are not located inside chloroplasts. Expressed differently, if the plastidic factor is present it leads to a suppression of gene expression. It appears that the selected proteins are located in the cytoplasm. $AsAT_3$ also showed this kind of dependence, but only to a small extent.

Two isoforms $AlAT_{1+2}$, were affected by the plastidic signal. If it is missing from the beginning of seedling development an increase in enzyme level does not take place. Apparently there is a positive correlation between the presence of the plastidic factor and the rise in the enzyme level. As a plastidic localization can be excluded, no decrease in $AlAT_1$ or $AlAT_2$ is detectable in the transition experiment; a localization outside the chloroplast but inside an organelle with a close relation to the plastid is suggested. A localization inside the peroxisomal compartment appears probable, which is in agreement with previous findings [7,23,26].

The separation of aminotransferases, AsAT and AlAT, into their isoforms shows their different regulation. AsAT is regulated only slightly with small modulations of activity, which could be lead back to isoform 3. This showed a certain dependence on negative control by the plastidic factor. On the other hand, the AlAT level is strongly regulated by light and the plastidic factor. The latter affects different isoforms differently, namely negatively in the case of $AlAT_3$, which represents a new phenomenon, and positively in the case of $AlAT_{1+2}$.

The complex relationships described can only be understood if separation into isoforms with a high recovery becomes possible. An important goal for the future is to confirm the "double character" of the plastidic factor, which was discovered recently by Schuster and Mohr [29], in the case of the two NiR isoforms in mustard cotyledons. The present results support these findings.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (SFB 206). The author is greatly indebted to the Studienstiftung des Deutschen Volkes for a scholarship, and thanks Professor H. Mohr for his steady support during this work and Professor R. L. Nicholson (Purdue University, West Lafayette, IN, USA) for assistance with the English.

REFERENCES

1 T. ap Rees, in D. D. Davies (Editor), *The Biochemistry of Plants*, Vol. 12, Academic Press, New York, 1987, Ch. 3, p. 87.
2 H. Mohr, *Der Stickstoff*, Springer, Berlin, 1990, p. 305.
3 R. J. Ireland and K. W. Joy, in P. Christen and D. E. Metzler (Editors), *Transaminases*, Wiley, New York, 1985, Ch. 6B, p. 376.
4 C. V. Givan, in B. J. Miflin (Editor), *The Biochemistry of Plants*, Vol. 5, Academic Press, New York, 1980, Ch. 8, p. 329.
5 F. Wightman and J. C. Forest, *Phytochemistry*, 17 (1978) 1455.
6 H. Thomas, *Z. Pflanzenphys.*, 74 (1975) 208.
7 S. Biekmann and J. Feierabend, *Biochim. Biophys. Acta*, 721 (1982) 268.
8 J. K. Bryan, in B. J. Miflin and P. J. Lea (Editors), *The Biochemistry of Plants*, Vol. 16, Academic Press, New York, 1990, Ch. 5, p. 161.
9 M. Gazeu-Reyjal and J. Crouzet, *Phytochemistry*, 15 (1976) 1619.
10 H. Thomas and J. L. Stoddart, *Annu. Rev. Plant Physiol.*, 31 (1980) 83.
11 F. J. Vences, F. Vaquero, A. M. Vazquez and P. De La Vega, *J. Plant Physiol.*, 132 (1986) 31.
12 H. Mohr, *Planta*, 49 (1957) 389.
13 H. Mohr, *Z. Pflanzenphysiol.*, 54 (1966) 63.
14 T. Reiss, R. Bergfeld, G. Link, W. Thien and H. Mohr, *Planta*, 159 (1983) 518.
15 H. Mohr and H. Drumm-Herrel, in H. Smith (Editor), *Plants and the Daylight Spectrum*, Academic Press, New York, 1981, p. 423.
16 W. D. Loomis, J. D. Lile, R. P. Sandstrom and A. J. Burbott, *Phytochemistry*, 18 (1979) 1049.
17 M. Hørder and R. Rej, in J. Bergmeyer and M. Grassl (Editors), *Methods of Enzymatic Analysis*, Vol. 3, Verlag Chemie, Weinheim, 3rd ed., 1983, Ch. 6.2, p. 444.
18 R. Rej and M. Hørder, in J. Bergmeyer and M. Grassl (Editors), *Methods of Enzymatic Analysis*, Vol. 3, Verlag Chemie, Weinheim, 3rd ed., 1983, Ch. 6.1, p. 416.
19 M. W. Neal and J. R. Florini, *Anal. Biochem.*, 55 (1973) 328.
20 U. Hecht, R. Oelmüller, S. Schmidt and H. Mohr, *Planta*, 175 (1988) 130.
21 C. Schuster, S. Schmidt and H. Mohr, *Planta*, 177 (1989) 74.
22 S. Schmidt and H. Mohr, *Planta*, 177 (1989) 526.
23 R. Oelmüller, *Phytochem. Phytobiol.*, 49 (1989) 229.
24 R. Oelmüller, I. Levitan, R. Beryfeld and H. Mohr, *Planta*, 168 (1986) 482.
25 A. Batschauer, E. Mösinger, K. Kreuz, J. Dörr and K. Apel, *J. Biochem.*, 154 (1986) 625.
26 T. Noguchi and S. Hayashi, *Biohem. J.*, 195 (1981) 235.
27 R. Weimar and G. M. Rothe, *Physiol. Plant.*, 69 (1986) 692.
28 P. Gegenheimer, *Methods Enzymol.*, 182 (1990) 174.
29 C. Schuster and H. Mohr, *Planta*, 181 (1990) 327.