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AMINO ACID METABOLISM IN THE PEDICEL–PLACENTA–CHALAZAL REGION OF THE DEVELOPING MAIZE KERNEL

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Key Word Index—*Zea mays*; Gramineae; maize; pedicel–placenta–chalazal region; amino acid metabolism.

Abstract—The pedicel–placenta–chalazal (PPCh) region is a heterogeneous maternal tissue located at the base of the endosperm. Its active role in the transport and metabolism of nitrogen compounds supplied to the endosperm has been shown by incorporation of labelled compounds, as well as by analysis of appropriate enzyme activities and the free amino acid pool. Within 30 min incubation, 60–70% of the label given in [^{14}C]aspartate or [^{14}C]glutamate was recovered in the organic acid fraction of the PPCh. Although the dry matter and protein content of the PPCh region was practically unchanged between days 15 and 30 after pollination, glutamate transaminase, alanine transaminase, NADP-malic enzyme and NAD-malate dehydrogenase were increased substantially. The PPCh region contains glutamine at a significantly higher concentration and alanine at a lower concentration than the endosperm. In contrast to glutamate synthase, the activity of glutamine synthetase per endosperm and per mg protein is significantly higher in the PPCh region. This implies involvement of these tissues in the transport of nitrogen compounds, mainly in the form of glutamine, as well as possible compartmentation of the glutamine–glutamate cycle in the developing maize kernel.

INTRODUCTION

The pedicel–placenta–chalazal (PPCh) region located at the base of the endosperm is a heterogeneous maternal tissue connecting the maize kernel with somatic parts of the plant [1]. Both anatomical and radioautographic studies indicate that this tissue is the only pathway through which assimilates enter the developing endosperm [2, 3]. Since there are no plasmodesmal connections between PPCh and the basal endosperm, PPCh forms a barrier which might be responsible for the translocation of metabolites against the concentration gradient [2]. Shannon reported on PPCh involvement in the transport of carbohydrates [4] and our preliminary data suggested its active role in amino acid metabolism [5].

The supply of nitrogen compounds is known to affect mainly the synthesis of storage proteins, i.e. zein and glutelins [6], which is closely correlated with the accumulation of carbohydrates in the kernel [7]. Although the biochemical mechanisms of both synthetic processes have been generally elucidated, the coordinate regulation of these is still unknown. Changes in the rate of accumulation of dry weight and reduced nitrogen in the ear and vegetative parts of the maize plant suggest that the availability of reduced nitrogen compounds, rather than that of carbon assimilates, limits kernel development [8]. On the other hand, it has been shown [9] that nitrogen compounds available for the synthesis of storage proteins are not fully utilized and that some regulatory mechanisms must be involved in the translocation of nitrogen from the vegetative parts to the developing kernels. It appears that PPCh tissue may be involved in these mechanisms.

RESULTS AND DISCUSSION

The amino acid metabolism in the PPCh region and endosperm was studied in the period of most intensive synthesis of storage proteins in maize kernel, i.e. between the 15th and 30th day after pollination (d.a.p.). The developmental stages of maize kernels on the 15th, 20th and 25th d.a.p. correspond to shoot apex stages 1, 3 and 4, as classified by scanning microscopy according to Abbe and Stein [10]. At the beginning of the experimental period (15 d.a.p.), PPCh constitutes *ca* 40% of the total dry matter of the kernel, and at the end (30 d.a.p.) only *ca* 7%.

Under our experimental conditions between the 15th and 30th d.a.p., the dry weight of endosperm was increased *ca* 10-fold, with a concomitant 3-, 15- and 50-fold increase per kernel in soluble proteins, glutelins and zein, respectively (Figs. 1 and 2). At the same time, for the PPCh region, both the weight and protein content per kernel remained practically the same, indicating full development of this tissue before the onset of intensive growth of the endosperm.

During the whole experimental period, the total pool of free amino acids in PPCh decreased only from 102 to 83 mM whereas that of the endosperm decreased by 147 to 37 mM. Similarly, the composition of the pool in PPCh did not change much, in contrast to the endosperm where participation of proline and alanine in the pool decreased from 13 to 1% and from 30 to 15%, respectively.

As shown previously by the paired data *t*-test [5], the participation in the free amino acid pool of glutamine and glutamate was significantly higher and of aspartate, serine and glycine significantly lower in PPCh than in the cob vascular sap of plants 15 d.a.p. The most striking quanti-

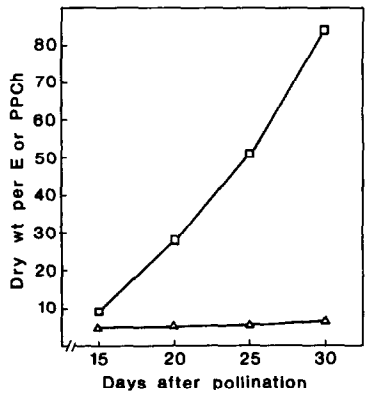


Fig. 1. Dry weight of the endosperm (E) (□) and PPCh (△) during development of maize kernel.

tative differences concerned glutamine, which in the sap constituted 25% [5, 11] and in PPCh up to 50% of the total free amino acid pool (Fig. 3). As shown in Fig. 3, the high contribution of glutamine to the free amino acid pool in PPCh persisted during kernel development until the 30th d.a.p.

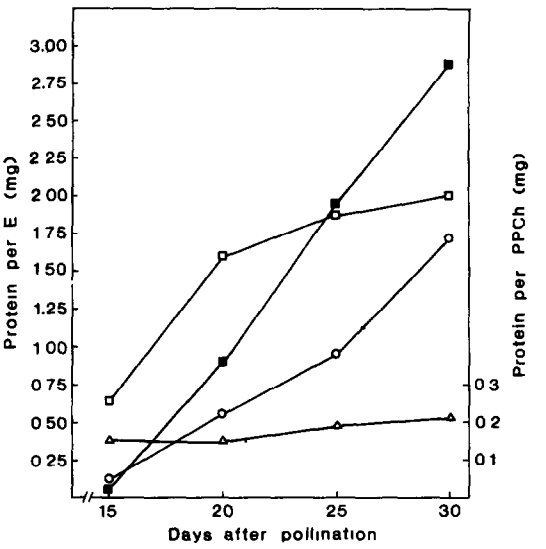


Fig. 2. Synthesis of zein (■), glutelins (○) and soluble proteins (□) in the endosperm (E) and in the PPCh (△) during development of maize kernel.

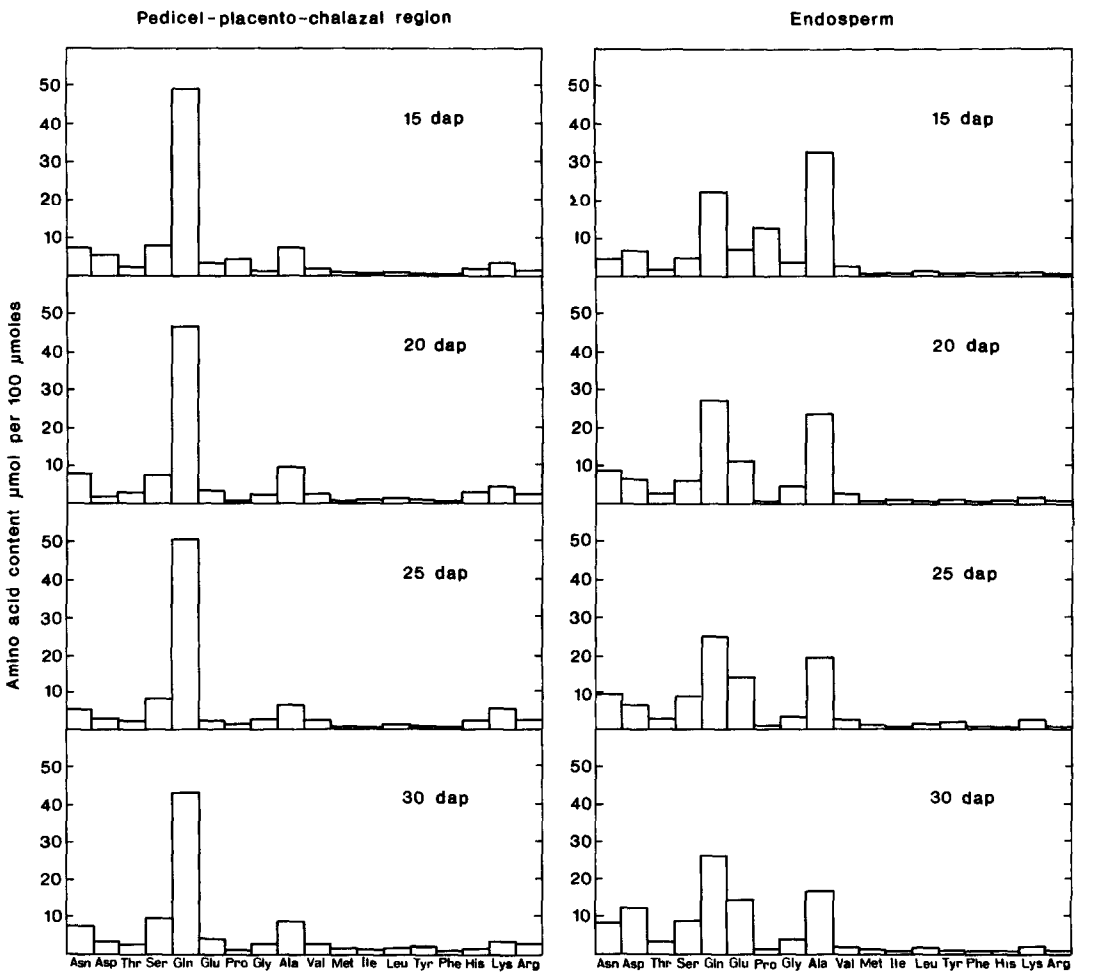
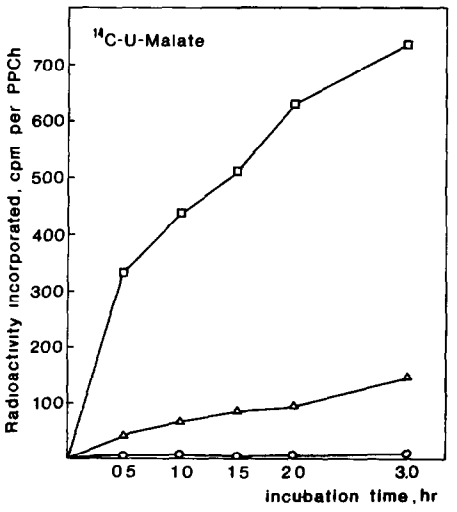


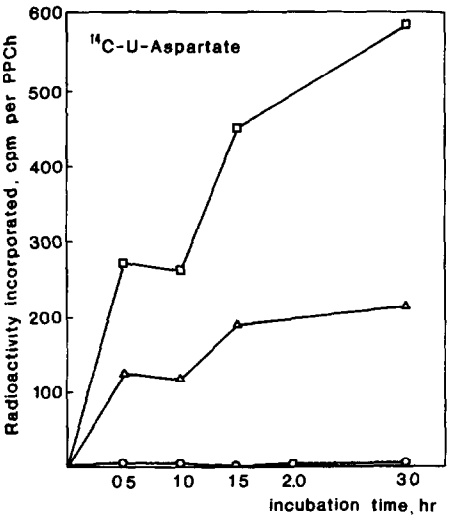
Fig. 3. Composition of the free amino acid pool in the PPCh and endosperm of the developing maize kernel.

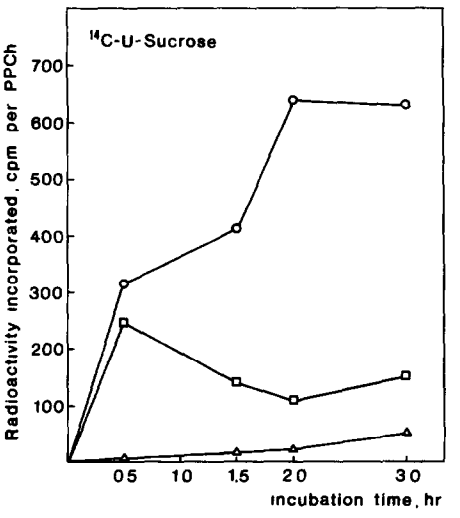
The concentration of glutamate and alanine throughout the whole period did not exceed 10% of the glutamine content. Participation of alanine and glutamate in the free amino acid pool was much higher for the endosperm than for PPCh; for glutamate, the difference amounted to 3- to 5-fold. The relative content of aspartate and asparagine did not exceed 10% of all the free amino acids.

Differences observed in the composition of the free amino acid pool in the cob vascular sap, PPCh and the endosperm [5] suggested transformation of amino acids in PPCh. This was confirmed experimentally by incorporation of uniformly labelled aspartate and glutamate into PPCh and measurements of the ^{14}C-label distribution in the acidic, basic and neutral fractions of the ethanol-soluble compounds of this tissue. For comparative purposes, [^{14}C]sucrose and [^{14}C]malate were included in these experiments. Translocation of sucrose through the kernel base has already been studied by Shannon [4]. The reason for inclusion of malate was that in maize, a C-4 plant of 'malate type', it is one of the major organic acids both in the vascular sap and in PPCh. On the 15th d.a.p., the malate concentration increased to 22.5 mM in the cob vascular sap and to 13 mM in PPCh (Łyżnik, unpublished results). Under our experimental conditions, after 30 min of incubation the radioactivity found in the alcohol-soluble fraction of PPCh constituted 90% of the total radioactivity incorporated and after 3 hr of incubation it decreased only by 5%. These results are in accordance with the data obtained by Shannon [4] with sucrose, pointing to a rapid uptake of metabolites by PPCh and their comparatively slow translocation to the endosperm.

The results presented in Fig. 4 show that aspartate was metabolized to organic acids more rapidly than glutamate, and that within 30 min, 70% of the label was recovered in this fraction. It is also of interest that irrespective of the absolute increase in ^{14}C-incorporation, the rate of aspartate transformation to organic acids remained constant during prolonged incubation, i.e. the percentage of radioactivity in the fraction retained on the Dowex-1 column remained practically the same. This indicates that in PPCh, as in legume seeds, aspartate was catabolized very rapidly to organic acids [12] and the conversion of this amino acid to asparagine was negligible or slow. As established previously [13], the main reason







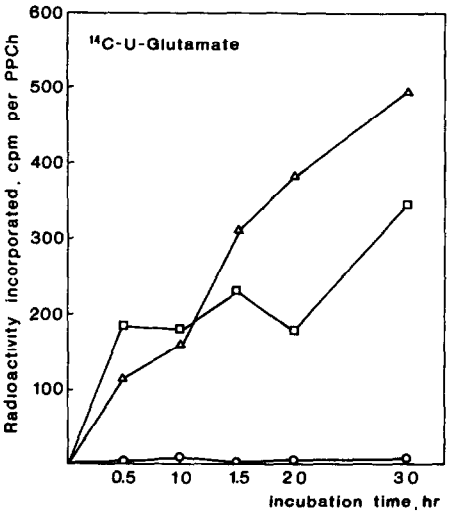


Fig. 4. Incorporation of ^{14}C-label from [^{14}C]aspartate, [^{14}C]glutamate, [^{14}C]malate and [^{14}C]sucrose into acidic (□), basic (△) and neutral (○) fractions of alcohol-soluble compounds of the PPCh region.

for the low conversion of aspartate into asparagine is its rapid transformation to oxaloacetate, which enters the tricarboxylic acid cycle. The time course of incorporation of the ^{14}C-label derived from glutamate was different from that from [^{14}C]aspartate. Transformation of glutamate into the carboxylic acid fraction was somewhat lower during the first 30 min, and on further incubation the label reappeared in the basic fraction consisting mostly of amino acids. This implies the synthesis of other amino acids by transamination processes and/or the formation of glutamine in a reaction catalysed by glutamine synthetase.

The appearance of the ^{14}C-label from malate in the basic fraction (up to 15% of the total radioactivity) suggests direct or indirect utilization of malate for the synthesis of amino acids in PPCh. The conversion of sucrose to amino acids, as judged from the rate of ^{14}C-label appearance, was half that of malate. The decrease in the initially high radioactivity in the organic acid fraction following introduction of [^{14}C]sucrose might be partly connected with the transformation of sucrose into amino acids in PPCh.

The assay of the enzymes metabolizing amino acids in PPCh and endosperm included GS, GOGAT and the transaminases GOT and GPT. Both the NAD- and NADP-dependent GDH activities were practically undetectable (< 5 pkat/mg protein) in either tissue and the activity of asparagine synthetase was one order of magnitude lower than that of glutamate synthase.

The specific activities of the enzymes given in Table 1 refer to kernels isolated 15 d.a.p. when zein in the endosperm was present in trace amounts and the content of glutelins did not exceed 15% of the total protein. The enzymatic activities of GOT and GOGAT in both tissues were similar whereas the GPT activity in the endosperm was about three times higher than that in PPCh. It should be emphasized that the specific activity of GS in PPCh was about 10-fold higher than that in the endosperm. Also, both tissues exhibited high specific activities of MDH and a relatively high one of ME. It is noteworthy that the specific activities of the malate-metabolizing enzymes at the onset of zein synthesis were higher in the PPCh than in the endosperm. ME is involved in photosynthetic carbon metabolism, and in photosynthesizing organs it is localized exclusively in the chloroplasts of the bundle sheath cells [14]. In non-photosynthesizing organs, both enzymes are responsible for the formation of substrates crucial both for anabolic and catabolic processes. It appears that these processes also take place in the PPCh and that this region is not exceptional among other non-photosynthesizing tissues.

Table 1. Specific activities of enzymes in the PPCh and endosperm on the 15th day after pollination

Enzyme	Activity (nkat/mg of protein)	
	PPCh	Endosperm
Glutamate-oxalacetate transaminase	14.2	16.3
Glutamate-pyruvate transaminase	8.9	27.2
Glutamate synthase	0.083	0.055
Glutamine synthetase	2.1	0.19
NADP-malic enzyme	0.86	0.40
Malate dehydrogenase	160	123

Following pollination, the activities of the enzymes investigated, calculated per single developing kernel, showed a gradual increase both in the PPCh and the endosperm (Figs. 5 and 6). This is inconsistent with the view of Felker and Shannon [2], who believed, on the basis of anatomical studies, that as early as on the 20th d.a.p. the cytoplasm of placenta–chalazal cells is disorganized and the cells are dead. Our enzymatic studies showed full metabolic activity of this region, at least up to the 30th d.a.p.

Due to the increasing contribution of endosperm tissues in the development of maize kernel, all enzyme activities studied except GS and ME, calculated per kernel, were distinctly higher in the endosperm than in the PPCh. The increase in the GOT, GPT, GOGAT and MDH activities in the endosperm from the 15th to 30th d.a.p. was 7-, 15-, 6- and 4-fold, respectively. The corresponding increase for the PPCh was 2- and 3-fold for GOT and GPT, 2-fold for MDH and practically negligible for GOGAT. However, taking into account the almost constant protein and dry matter content of the PPCh during kernel development, it is evident that amino acid metabolism in the PPCh was accelerated during the synthesis of storage proteins in the endosperm. Other authors similarly observed an increase in the activity of nitrogen-assimilating enzymes in the endosperm at the initiation of zein synthesis [15, 16].

Since GDH activity was below the detectable limit in the PPCh and the endosperm, it is believed that the glutamine–glutamate cycle, catalysed in a coordinate manner by GS and GOGAT, is the main route of nitrogen assimilation of the developing kernel. This is consistent with the earlier findings of Sodek and da Silva [15]. In this context, considerable interest is attached to the very high specific and total GS activity in PPCh. As shown in Fig. 6, the GS activity per kernel increased during kernel development and on the 30th d.a.p. it was twice as high in the PPCh than in the endosperm. Contrary to this, GOGAT remained practically at the same level in the PPCh while in the endosperm it increased *ca* 6-fold. The repeated transient depression (by *ca* 30%) of GS activity both in the PPCh and the endosperm is not yet understood.

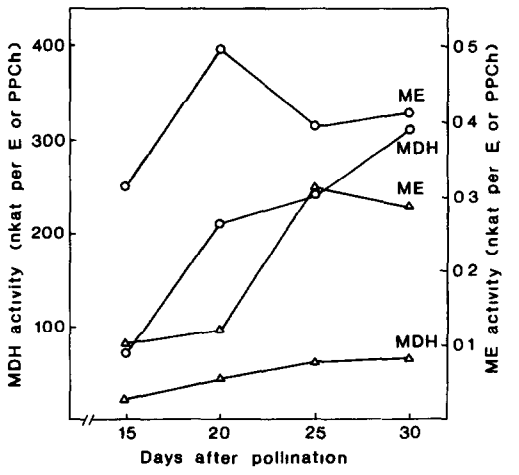


Fig. 5. Activity of malate dehydrogenase (MDH) and NADP 'malic enzyme' (ME) in the PPCh (△) and endosperm (E) (○).

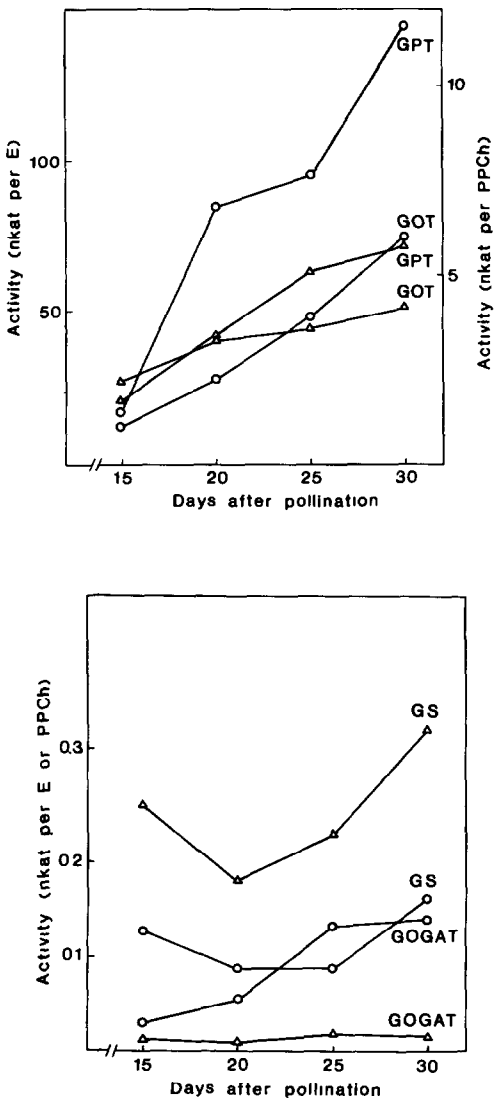


Fig. 6. Activity of amino acid metabolizing enzymes in the PPCh (△) and endosperm (E) (○): glutamate transaminase (GOT), alanine transaminase (GPT), glutamine synthetase (GS) and glutamate synthase (GOGAT).

The foregoing results drew our attention to the Gln content and its metabolism. A very high concentration of free Gln was characteristic of the PPCh, where it constituted *ca* 50% of the total free amino acid pool. It exceeded the Gln content in all the other maize tissues examined: leaf (3%), stem (17%), hypsophyll (15%), cob vascular sap (20%) and endosperm (25%) [5, 11, 17]. At the same time, the Ala/Gln ratio, which in the leaf equalled 20, decreased to 3 in the stem and to 0.3 in PPCh, but rose to 1 in the endosperm. Similarly, the Glu/Gln ratio of *ca* 3 in the leaves, equalled only 0.1 in PPCh and increased to *ca* 2 in the endosperm.

The amino acid ratios in the free amino acid pool and the GS/GOGAT ratio in the PPCh and the endosperm during kernel development throw some light on Gln metabolism and the role of the PPCh in the synthesis of storage proteins in maize. The activity of GOGAT in the PPCh was very low and throughout the whole experimental period the GS/GOGAT ratio was as high as 20. In the endosperm this ratio was *ca* 4 on the 15th d.a.p. and decreased to *ca* 1 when measured 30 d.a.p. Antonielli *et al.* [17] compared the ratio of GS to GOGAT and the level of Gln in different maize tissues and came to the conclusion that the ratio of 7 found in leaf lamina is optimal for the glutamine–glutamate cycle and the assimilation of nitrogen to function. There are no data available for the storage tissues but the high GS/GOGAT ratio in the PPCh with a concomitant high level of Gln suggests that this region is the main site of Gln synthesis in maize kernel and it is responsible for transportation of nitrogen compounds to the endosperm. However, it appears that the second part of the glutamine–glutamate cycle, catalysed by glutamate synthesis, takes place mainly in the endosperm. Gln formed in the PPCh could be utilized in the endosperm both directly for zein synthesis and for the synthesis of other amino acids by GOGAT and transaminases. The high activity of these enzymes in the endosperm resulted in the increased concentration of Glu and especially of Ala. Also it is of interest that during kernel development the Ala/Glu ratio in the PPCh increased from 0.9 to 2.5 but at the same time decreased from 4 to 1.5 in the endosperm. This implies the use of glutamate synthesized in the GOGAT reaction for the synthesis of other amino acids.

The high GS activity in the PPCh and the high GOGAT activity in the endosperm suggest compartmentation of the nitrogen assimilation process in maize kernel. Data on the concentration of the NH_4^+ ion and of amino acid substrates and on their affinities for GS and GOGAT would provide information on the extent to which this compartmentation performs a regulatory role in zein synthesis.

EXPERIMENTAL

Plant material. W64A maize (*Zea mays* L.) inbred was grown in soil in a greenhouse under normal cultivation conditions. All plants were self-pollinated. Ears were harvested on the 15th, 20th, 25th and 30th d.a.p. Endosperms and PPCh regions were separated from the kernels and frozen in liquid N_2 immediately after removal from the middle part of the ear. Determinations of free amino acids, protein fractions and dry wt were performed on the lyophilized material.

^{14}C-labelling experiments. Kernels removed from the ears 20 d.a.p. were incubated at 30° for the time indicated, in a medium containing inorganic nitrogen, metal ions, micro-elements and sucrose according to ref. [18], and in addition 20 mM malate and the 10 dominant amino acids, at concns found in the cob vascular sap (cf. ref. [5]). Uniformly labelled [^{14}C]aspartate, [^{14}C]glutamate, [^{14}C]malate and [^{14}C]sucrose were supplied to the medium at concns of 4 mM (7.0 kBq/ml), 1.2 mM (5.5 kBq/ml), 20 mM (8.2 kBq/ml) and 150 g/l. (5.4 kBq/ml), respectively. The kernels were mounted so that only their basal parts (PPCh) were immersed in the medium. After incubation, the endosperm and PPCh tissues were separated, homogenized in 80% EtOH and centrifuged at 10000 *g* for 10 min. The resultant supernatant was evapd to dryness and the residue was dissolved in H_2O. One part of the supernatant was used for determination of total radioactivity and the other was passed through conjugated columns of Dowex W × 8 (H^+ form) and Dowex × 8 (formate form). Amino acids were eluted with 2 M NH_3 and organic acids with 6 M HCO_2H. After evapn of the collected

fractions, the residues were dissolved in a small amount of H_2O and the radioactivity incorporated into acidic, basic and neutral fractions was measured using Bray's scintillation liquid.

Protein fractionation. Endosperm proteins were fractionated according to ref. [19].

Determination of free amino acids. Amino acids were determined using a Beckman amino acid analyser. The content of asparagine and glutamine was calculated from the difference in the content of aspartate and glutamate before and after mild acid hydrolysis as described previously [5].

Enzyme extraction and assay. Extracts for enzyme analysis were prepared by grinding 30 endosperms or 50 PPCh tissues frozen in liquid N_2 with 3 ml 0.1 Tricine buffer (pH 7.8) containing 10 mM 2-mercaptoethanol, 1 mM $MgCl_2$ and insoluble PVP (20 g/l.) in a chilled mortar. Homogenates were centrifuged for 20 min at 10000 *g* at 4°. Low-M_r compounds were removed from the supernatant using Sephadex G-25 coarse columns. Tricine buffer (pH 7.8) proved the most universal for the enzymes tested. Glutamate synthase was assayed spectrophotometrically with NADH according to ref. [15] except that 67 mM Tricine was used instead of Tris–HCl buffer and EDTA was at 1.1 mM concn. The assay of glutamine synthetase was based on the formation of γ-glutamylhydroxamate [20]. The reaction mixture contained: 50 mM Tricine buffer pH 7.8, 10 mM $MgCl_2$, 5 mM ATP, 50 mM glutamate, 10 mM NH_2OH, 40 mM KCl, 0.5 mM EDTA and 1.2 mM 2-mercaptoethanol. After incubation (15 min at 30°) the reaction was stopped by the addition of 2 ml of the $FeCl_3$ reagent. NADP-dependent malic enzyme was assayed as in ref. [21]. The reaction mixture consisted of 67 mM Tricine (pH 8.2), 0.3 mM EDTA, 3.3 mM malate, 0.8 mM NADP and 6.7 mM $MgCl_2$; the latter served to initiate the reaction. The activity of NAD-dependent malate dehydrogenase was determined according to ref. [19]. Alanine transaminase was assayed spectrophotometrically at 340 nm. The reaction mixture contained 67 mM HEPES (pH 7.25), 8 mM 2-oxoglutarate, 17 mM alanine, 0.2 mM NADH, 5 mM 2-mercaptoethanol, 0.025 mM pyridoxal phosphate, 3.3 mM EDTA and 0.46 μkat LDH. Glutamate transaminase was measured spectrophotometrically at 340 nm. The reaction mixture contained 67 mM Tricine (pH 7.8), 4 mM 2-oxoglutarate, 17 mM aspartate, 0.2 mM NADH, 5 mM 2-mercaptoethanol, 0.025 mM pyridoxal phosphate and 3.3 mM EDTA. Asparagine synthetase was assayed according to ref. [12]. Glutamate dehydrogenase was determined spectrophotometrically at 340 nm in the system described in ref. [22].

Determination of protein. Protein content was determined according to ref. [23], using bovine serum albumin (fraction V) as the standard.

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