nine in chick diets. A test feeding of a diet containing the related compound, DL-homocysteine thiolactone hydrochloride, showed that this substance was only about 75% as effective as methionine, DL-homocystine, and DL-homocysteine.

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Metabolism of the Amino Group of Glutamate in Maize Inbred Lines and Their F_1 Hybrid after Infiltration of α-Ketoglutarate and ^{15}N-Labeled Ammonium Sulfate

Yordanka I. Mladenova

Vacuum infiltration of α-ketoglutarate and $(^{15}NH_4)_2SO_4$ was carried out in excised leaves of plants from maize inbred lines and their F_1 hybrid at the sixth true leaf phase. It was established on the basis of the ^{15}N enrichment of the free and protein glutamate, and of the remaining free and protein amino acids, that the processes connected with the operation of the α-ketoglutarate–glutamate system are more intense in the hybrid than in the parent inbred lines. The processes involved are: the reductive amination of α-ketoglutarate, the transamination of glutamate with the remaining amino acids, and the oxidative deamination of glutamate.

The studies described here constitute continuation of our previous work during which differences were established between maize inbred lines and their F_1 heterotic hybrids as regards the changes in the content of some of the free amino acids following infiltration of keto acids and ammonium sulfate in leaf tissue (Mladenova and Istatkov, 1968). A change in the glutamate content, depending on the genotype and on the development phase of the plants, was established under these conditions.

Heavy nitrogen (^{15}N) was used as an indicator in order to obtain indications of the movement of the amino group in processes related to the operation of the α-ketoglutarate–glutamate system after vacuum infiltration of α-ketoglutarate and [^{15}N]ammonium in excised leaves of different maize genotypes.

MATERIALS AND METHODS

Plants used were of the maize inbred lines W-32 and W-187, and their heterotic hybrid W-32 × W-187 cultivated in a greenhouse, at the sixth true leaf phase. The seeds were planted in pots filled with quartzite, and the nutrient solution of Hoagland-Arnon I was used. Vacuum infiltration was carried out by folding 5 g of fresh leaves in gauze packets and by placing them in a beaker containing a 1:1 solution of 0.05 *M* α-ketoglutarate and 0.05 *M* $(^{15}NH_4)_2SO_4$ with a ^{15}N abundance of 12.7 atom %. The beaker was placed in a vacuum dessicator, and vacuum treatment was continued until no more air bubbles appeared. Then the vacuum was gradually released. After infiltration, the samples were washed in distilled water and placed at room temperature in a humid glass chamber. They were inactivated at 105° for 5 min at intervals of 15 and 60 min and then dried to a constant weight; 300 mg was taken from the dried and ground material in order to prepare the amino acid extracts. For the free amino acids, the samples were placed in 80% ethanol, followed by a water-bath extraction. After decantation, the alcohol was evaporated and the dry residue was dissolved in 1 ml of water. After filtration, the filtrate and the rinsing water were passed through cation-exchange resin KPS (in H^+ form).

Electrophoretic separation was carried out with purified amino acid solutions for 40 to 50 min at 500 V, using 0.1 *M* pyridine–acetic acid buffer (pH 5.4). A ninhydrin solution was used for the visualization of the amino acid bands on the electropherograms. After extraction of the free amino acids, the protein amino acids were obtained after hydrolysis of 100 mg of the ground residue previously dried at 65°. Hydrolysis was carried out with 6 *N* HCl for 48 hr at 110°. The free and protein amino acids separated under the influence of the electric field were grouped in the following

Institute of Genetics and Plant Breeding, Sofia, Bulgaria.

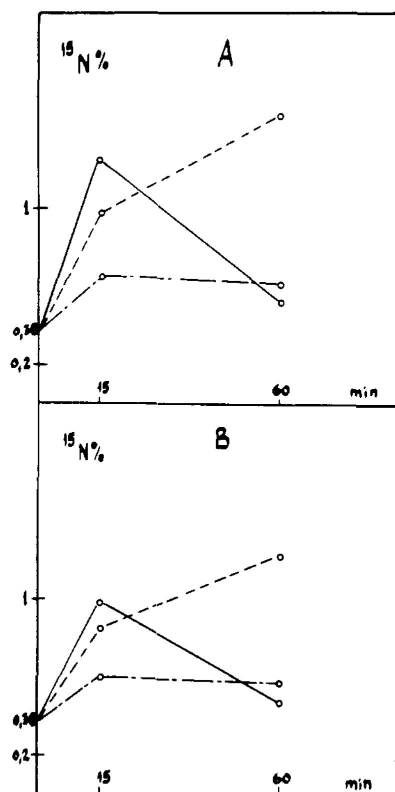

Figure 1. Amount of ^{15}N in the free glutamate (A) and free amino acids, basic + neutral + aspartate (B), after vacuum infiltration of 0.05 *M* α-ketoglutarate and 0.05 *M* $(^{15}NH_4)_2SO_4$ (labeled with 12.7 atom % ^{15}N) in excised leaves of maize inbreds: W-32 (-·-); W-187 (- - -); and their F_1 hybrid W-32 × W-187 (—). (Abscissa) exposure (in minutes) after the infiltration; (ordinate) ^{15}N abundance (in atom percent) of amino acids; 0.38 atom % ^{15}N is the natural ^{15}N abundance.

manner: (A) free glutamate; (B) free basic amino acids, free neutral amino acids, and free aspartate; (C) protein glutamate; (D) protein basic amino acids, protein neutral amino acids, and protein aspartate. The amino acid bands cut from the electropherograms and divided into the above-mentioned groups were sliced into thin tapes and were eluted with 80% ethanol until complete discoloration occurred. The eluates of the amino acids from group B were pooled together, as were the eluates of the amino acids of group D. After evaporation of the alcohol from the above eluates, the nitrogen content of the amino acids was determined by the semimicro method of Kjeldahl. The samples were mineralized with mixed selenium catalyst, dissolved in hot concentrated sulfuric acid (Istatkov and Mladenova, 1970). The distilling ammonia was collected in 0.01 *N* sulfuric acid. The excess of acid was titrated with 0.01 *N* sodium hydroxide in the presence of a Tashiro indicator. After titration, the solution was acidified with several drops of 0.01 *N* sulfuric acid and evaporated over a water bath to the volume required to give a nitrogen concentration of 500 μg/ml. For the subsequent chemical processing 200 μl was used. The conversion of NH_4^+ into molecular nitrogen was carried out by the sodium hypobromite method in a high vacuum range (10^{-5} Torr). A NOI-3 ^{15}N analyzer was used for the optical spectroscopic isotope analysis of the molecular nitrogen. The separation of the isoenzymes of the NAD^+-dependent glutamate dehydrogenase was carried out by disc electrophoresis in 7.5% polyacrylamide gel (pH 8.9) (Davis, 1964). Details concerning the extraction of the enzyme, the separation of the isoenzymes, the staining procedure, and the recording of the isoenzyme spectra have been previously described (Mladenova and Nicolov, 1974).

The maximum deviation of the values of the nitrogen

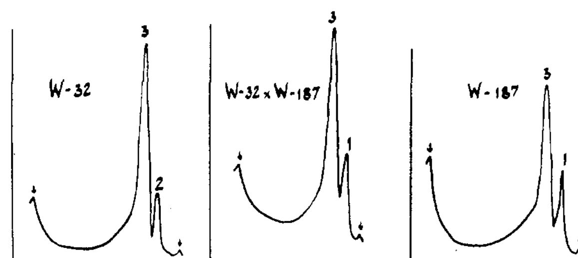

Figure 2. Densitograms (copies of densitograms of one of the three replications performed) of the isoenzymes of NAD^+-dependent glutamate dehydrogenase extracted from leaves of maize inbreds W-32, W-187, and their F_1 hybrid W-32 × W-187.

Table I. Area (cm^2) Surrounded by Peaks Outlined by Densitograms Which Show the Isoenzyme Spectra of NAD^+-Glutamate Dehydrogenase, Extracted from Excised Leaves of Plants of Inbred Lines of Maize and Their F_1 Hybrid

Genotype	Area, cm^2
W-32	4.36
W-187	3.99
W-32 × W-187	5.59

quantity of the free and protein amino acids was ±0.02%. The variations in the quantity of ^{15}N (in atom percent) for all replications were of the order of 2% relative error which was within the range of accuracy of the method. The semimicro-nitrogen analyses were carried out twofold in three replications. The emission spectrometric analyses were performed twofold in four replications. The electrophoretic analyses were conducted twofold in three replications.

The reliability of the differences of the values between the genotypes (presented in Figure 1 and Table I) was proven by the function $\psi^2(n,t)$ for accuracy factors of P = 5%, P = 1%, and P = 0.1% (Barov and Yovcheva, 1964).

RESULTS

The ^{15}N enrichment of the glutamate, after the infiltration of α-ketoglutarate and $^{15}NH_4^+$, is higher in the hybrid than in the parental inbreds upon 15-min exposure (Figure 1A). It is known that 15 min after the beginning of NH_4^+ infiltration in roots of 5–6-day-old seedlings, induction of the NAD^+-dependent glutamate dehydrogenase begins to be apparent (Kretovich et al., 1969). The data in Figure 1A lead us to assume that the process of reductive amination of α-ketoglutarate, 15 min after the beginning of infiltration of [^{15}N]ammonium and α-ketoglutarate, is the most intensive one in the hybrid. This fact is based on other studies in which differences were established between the lines and the hybrids as regards the isoenzyme spectra of NAD^+-glutamate dehydrogenase isolated from the leaves of the plants. It was established that the three genotypes differ both in relation to the qualitative composition of the isoenzyme spectra and in relation to the staining intensity of the bands. These differences are shown in Figure 2 and in Table I. Obviously the area closed by the isoenzyme peaks outlined on the densitograms, which is an indication of the degree of total activity of the glutamate dehydrogenase, is greatest in the hybrid. This fact was confirmed by studying two more hybrids and their parent inbreds (Mladenova, 1975). It concerns the reversible reaction of oxidative deamination of the glutamate, catalyzed by the same enzyme (L-glutamate:NAD(NADP) oxidoreductase (deaminating), EC 1.4.1.3) which catalyzes the reductive amination of glutamate to α-ketoglutarate. The activity of that enzyme was used as a supplementary criterion for the activity of operation of the system studied.

The above-mentioned data suggest that the system of α-ketoglutarate–glutamate operates more intensely in the hybrid than in the inbred lines, both in direction of the synthesis of glutamate and of the oxidation of glutamate to α-ketoglutarate. In most cases the nitrogen atoms required for the amino acid formation are limited in origin; they are most frequently the N atoms of the α-amino groups of the glutamate and aspartate or the acid–amido groups of the asparagine and glutamine. Taking this fact into account, the highest ^{15}N enrichment of all free amino acids (the glutamate excluded) observed 15 min after the infiltration in the hybrid (Figure 1B) could be explained by more intense processes of transamination of the glutamate with the remaining amino acids in the hybrid than in the inbred lines. Furthermore, it is possible that the enzyme amination of the γ-carboxylic group of the glutamate, which obtains glutamine, occurs more intensively in the hybrid than in the lines. Analyzing the results shown in Figure 1B, the possibility must also be kept in mind of synthesis of alanine, serine, glycine, and valine through the reductive amination of the respective keto acids (Kretovich, 1965), as well as the possibility of obtaining asparagine.

In the hybrid, it is possible to observe a sharp reduction of the ^{15}N enrichment of the free glutamate, 60 min after the infiltration (Figure 1A); this is also accompanied by a sharp decrease of the ^{15}N enrichment of the remaining free amino acids (Figure 1B). This could be explained by a more intense process of transdeamination in the hybrid than in the lines, a problem which will be dealt with later. It is well known that in aging organs, and particularly in organs separated from the plants (as is the case with the present studies), the reversible reaction by which glutamate is converted into α-ketoglutarate plays an important role in the oxidative deamination of the amino acids with formation of ammonia at the same time (Bonner and Varner, 1968).

The changes in the amount of ^{15}N in the amino acids of the hybrid, as shown in Figures 1A and 1B, could not be explained by intensified protein biosynthesis in the hybrid, as the ^{15}N enrichment (as far as ^{15}N atom percent excess is concerned) of the protein amino acids (those from groups C and D) of all three genotypes is not detected.

The fact that the decrease in the ^{15}N enrichment of the free glutamate in the hybrid 60 min after the onset of the infiltration is accompanied by a decrease in the ^{15}N enrichment of the remaining free amino acids as well (Figures 1A and 1B) is an indication that the glutamate undergoes oxidative deamination, with the parallel transamination of the remaining amino acids with α-ketoglutarate leading to the formation of glutamate. The latter, in turn, releases its nitrogen as ammonia, during its oxidative deamination, and is converted back into α-ketoglutarate, thereby closing a circle in the process and ensuring the easy and prompt deamination of all amino acids. Obviously, the combination of transamination and oxidative deamination of the glutamate operates at a higher activity in the hybrid than in the inbred lines.

What is the biological importance of this fact? Does it have any connection with the manifestation of hybrid vigor?

Actually, glutamate is the only amino acid which undergoes deamination in a comparatively intensive and energetically useful manner, but since the degradative reactions of the amino acids in the organism yield considerably lower coefficients of energy used, compared with the degradative reactions of the carbohydrates and triglycerides, the biological importance of the fact we established should not be sought in any energy advantages in this respect in the hybrid.

Our results show that the system of α-ketoglutarate–glutamate, a system of important strategic value, operates more actively in the hybrid than in the inbred lines. The greater activity of this system in the hybrid than in its parent inbreds could be in connection with the hybrid vigor manifestation, since it could lead to a more rapid filling-up of the amino acid pool in the cells of the hybrid than in those of the lines, in accordance with the needs of the protein synthesis and the organisms, respectively. Certainly it could be expected that not just one or the other level of metabolic activity would contribute to hybrid growth, but also the possibility of its being adequately regulated according to the needs of the entire metabolism. The investigations presented in this paper give some information on the differences between the genotypes only with regard to the "capacity" of the system of α-ketoglutarate–glutamate. Another question is when and how this capacity would be utilized and how it would contribute to the formation of one or other phenotype.

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