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Effect of L-Glutamic Acid and Siapton Leaf Organic Fertilizer on Oxidized Nicotinamide Adenine Dinucleotide Dependent Glutamate Dehydrogenase of Different Maize Genotypes

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Leaves of 14-day-old plants of inbred maize lines and their F_1 hybrid were vacuum infiltrated with solutions of L-glutamic acid (1.25×10^{-2} M, pH 6.67), Siapton leaf organic fertilizer (7 g L^{-1}, pH 6.27), and potassium sodium phosphate buffer (1.25×10^{-2} M, pH 6.58) as control. The molecular heterogeneity of nicotinamide adenine dinulcleotide–glutamate dehydrogenase (NAD^+–GDH) was determined by polyacrylamide gel electrophoresis. Differences were established between the investigated genotypes concerning changes in the isoenzyme spectra of NAD^+–GDH, caused by exogenous L-glutamic acid. It was established that the enzyme activity increases under the action of this acid in all three genotypes, an increase which is not due to increased H^+ ion concentration. The effect of L-glutamic acid on the deaminating activity of GDH in leaves of 14-day-old maize plants proved to be nonspecific. The Siapton leaf organic fertilizer, whose biologically active part is a mixture of free amino acids obtained after protein hydrolysis, has a similar effect.

The possibilities of regulating the action of glutamate dehydrogenase under the influence of different metabolites and physical agents have been investigated in animal tissues, microorganisms, bacteria (Frieden, 1963; Stadtman, 1966), and also more recently in higher plants (Kretovich et al., 1970, 1971, 1972; Barash et al. 1973; Bayley et al., 1972; Hartmann, 1973; King and Yung-Fan Wu, 1971; Pahlich, 1971, 1972; Pahlich and Hoffman, 1975; Sahulka et al., 1975; Sahulka and Gaudinova, 1976). The effects of adenylate metabolites, as well as some inorganic ions, growth regulators, herbicides, and amino acids, have been studied.

The effect of NH_4^+ ions and L-glutamic acid on changes in the isoenzyme spectra of nicotinamide adenine dinucleotide–glutamate dehydrogenase (NAD^+–GDH), extracted from roots of 6- and 14-day-old plants of maize inbreds and their F_1 hybrid, were studied in a previous work (Mladenova, 1977). Here we submit data on the effects of exogenously supplied L-glutamic acid and Siapton leaf organic fertilizer (a polypeptic amino acidic mixture, obtained after partial protein hydrolysis) on isoenzyme spectra of NAD^+–GDH extracted from leaves of 14-day-old plants of different maize genotypes. We assume that investigation of the effect of this fertilizer on the metabolism of different maize genotypes is expedient as our previous work (Mladenova and Dankov, 1976) showed that the effect of Siapton on the yield depended not only on the type of the culture but also on the genotype within the scope of a given culture.

MATERIAL AND METHODS

Fourteen-day-old plants hydroponically grown on Hoagland-Arnon I nutritive solution of the W-32 and W-187 inbred lines and of the F_1 hybrid W-32 × W-187 were investigated. They were cultivated in a growth chamber at 23 °C, 65–70% humidity, and 10 000-lx light intensity with 14-h "day" and 10-h "night". A solution of glutamic acid (1.25×10^{-2} M), potassium–sodium phosphate buffer with the same molarity (as control) and a Siapton solution (7 g L^{-1}) were infiltrated in 1 g of leaf mass under vacuum (10^{-2} Torr). The vacuum infiltration procedure has been described elsewhere (Mladenova, 1975). Solution pH was determined with a CP2 pH meter with glass and calomel electrodes. The enzyme was extracted (4 °C) by homogenizing 1 g of fresh material with 3 mL of 10^{-2} phosphate buffer (pH 7.4) containing 0.5 M sucrose and 6 mM ascorbate. The homogenization was performed as follows: the sample was put in a glass mortar, and then 1 mL from the above buffer and 0.5 g of ion-exchange resin Dowex 1 × 8 (200–400 mesh), equilibrated to pH 7.4 with the same buffer, were added. Next, the material was ground (3 min) and the remaining buffer (2 mL) was added, followed by final and full homogenization (2 min). After storing the samples for 1 h at 4 °C, followed by centrifugation (Janetzki K-24 centrifuge) with cooling (50 min, 18 000g), the clear supernatant was used to determine the protein content (Lowry, 1951) and for electrophoresis of 400 μg of protein. The isoenzymes were separated by means of disc electrophoresis in 7.5% polyacrylamide gel, pH 8.9 (Davis, 1964). The isoenzyme

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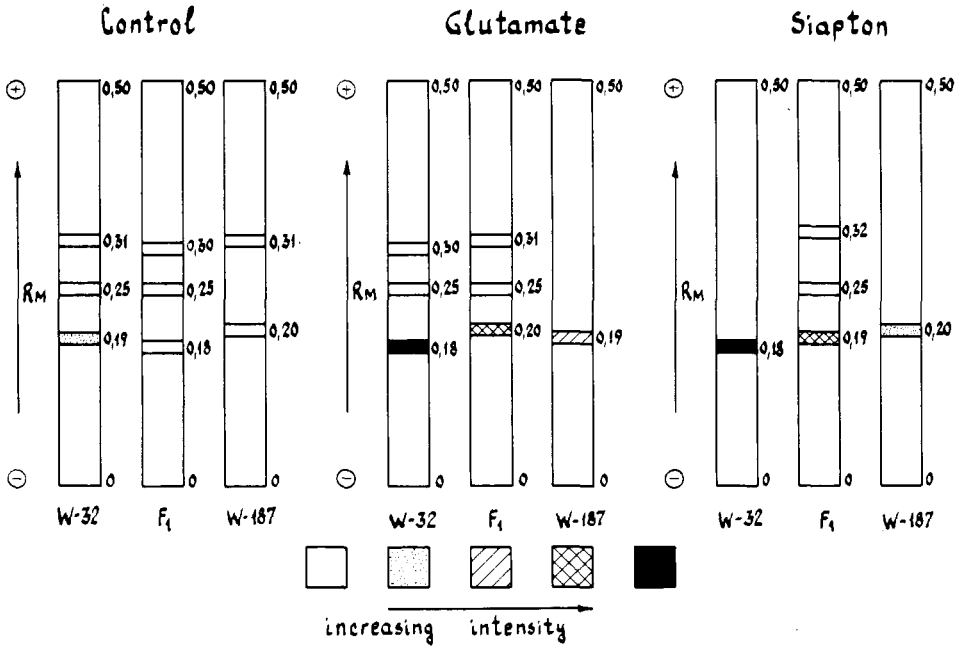

Figure 1. Schematic diagram to show the effect of L-glutamic acid and of Siapton leaf organic fertilizer on the isoenzyme spectra of NAD^+–GDH extracted from leaf tissue of 14-day-old plants of maize inbreds and their F_1 heterotic hybrid. Treatments: (1) infiltration in leaves of 1.25×10^{-2} M potassium sodium phosphate buffer, pH 6.58 (control); (2) infiltration in leaves of 1.25×10^{-2} M L-glutamic acid, pH 6.67; (3) infiltration in leaves of Siapton solution (7 g L^{-1}, pH 6.27).

zones were stained by forming formasans, as described by Gilmanov et al. (1967). The staining mixture without substrate was used as control. The isoenzyme bands were identified by means of relative electrophoretic mobility (RM) calculated on the basis of densitometric curves, obtained after direct densitometry of the gels with an ERI-65 densitometer. The total enzyme activity was evaluated by the area enclosed by the peaks of the densitometric curves. Four analyses were made in three replications per genotype. The reliability of the differences of values between the genotypes and between those of the individual treatments was investigated with the function $\psi^2(n,t)$ (for accuracy factors of $P = 0.1$, 1, and 5%, Barov and Yovcheva, 1964).

RESULTS AND DISCUSSION

It was established that the deaminating activity of glutamate dehydrogenase (EC 1.4.1.3) increases in all investigated genotypes under the influence of exogenous L-glutamic acid, but to a different degree (Figure 1). The increased enzyme activity is due chiefly to an increase in the activity of the molecular form with RM 0.18–0.19.

Moreover, it was established that the increase of NAD^+–GDH under the influence of L-glutamic acid was not due to an increase of H^+ ion concentration. An increase in enzyme activity was found although pH conditions in both treatments (glutamate and buffer) were kept within the limits of the same pH unit (pH 6.67 and 6.58). In our subsequent experiments we shifted the H^+ ion concentration of the control towards the acid region to pH 5.05 and 4.3. The same effect of increased enzyme activity was observed, although the H^+ ion concentration of the control was higher than in glutamate variant.

In the inbred W-187, besides a quantitative change in the enzyme activity, a qualitative change in the isoenzyme spectrum of NAD^+–GDH under the action of L-glutamic acid was also established, i.e., the disappearance of isoenzyme with RM 0.31.

An increase in NAD^+–GDH activity was also observed after infiltration in leaves of the Siapton leaf fertilizer (Figure 1).

Table I. Content of Free Amino Acids in Siapton Leaf Organic Fertilizer, Determined by Means of Automatic Amino Acid Analyzer (g %, w/w)

amino acid	g % (w/w)
L-aspartic acid	0.477
serine	0.163
L-glutamic acid	0.804
glycine	3.703
alanine	2.147
cystine	0.521
methionine	0.207
isoleucine	0.101
leucine	0.254
tyrosine	0.071
phenylalanine	0.183
γ-aminobutyric acid	0.017
tryptophan	0.149
ornitine	0.599
lysine	0.295
ammonia	0.418
histidine	(traces)
arginine	0.261
total (without NH_3)	9.952

According to data of the S.I.A.P.A. firm, this fertilizer contains, besides peptides, a mixture of free amino acids obtained after partial hydrolysis of animal proteins. The amino acid content of Siapton is relatively constant, showing slight changes in the single amino acid from batch to batch. As can be seen from Table I, the amino acid content is relatively low, but biological effect of this type of fertilizer is known specifically to be due to free amino acid participation (S.I.A.P.A.–CER, 1974). The treatment concentration (through spraying) recommended by the firm is 7 g L^{-1}. We used the same concentration for infiltration. From this concentration and that of the glutamic acid in the fertilizer (0.804%, Table I), it is possible to estimate the molarity of the glutamic acid introduced into the leaves by the Siapton solution. It can be assessed that this molarity is ca. 1000 times lower than that of the glutamic acid during the first series of experiments.

The table shows that the excess of the remaining amino acids in this balanced amino acid mixture is also very

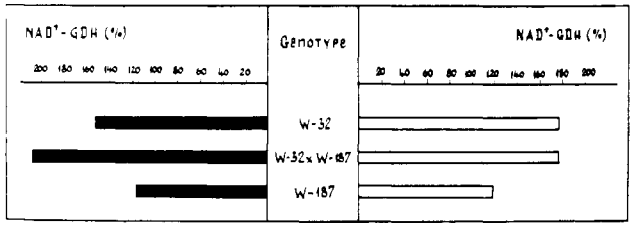

Figure 2. Activity of NAD$^+$–GDH after infiltration of 1.25×10^{-2} M L-glutamic acid (pH 6.67) (□) and of Siapton leaf organic fertilizer (7 g L^{-1}), pH 6.27 (■) in leaves of 14-day-old plants of F_1 hybrid W-32 × W-187 and its parental inbred lines. Control: infiltration of 1.25×10^{-2} M potassium sodium phosphate buffer, pH 6.58 (percent to the control).

small. For all that, however, data indicate (Figure 1) that the change in enzyme activity under the influence of the amino acid excess with the Siapton treatment is similar to the change of NAD$^+$–GDH activity under the action of exogenous L-glutamic acid in the first series of experiments.

Besides changes in activity, qualitative changes in the isoenzyme spectra of NAD$^+$–GDH after Siapton infiltration were observed. It concerns disappearance from the spectrum of the inbred W-32 of the molecular forms with RM 0.31 and 0.25 and of the component with RM 0.31 from the spectrum of the male parent W-187 (Figure 1).

Our data clearly illustrate that the nonspecific effect of exogenous amino acid interference on the activity of NAD$^+$–GDH conceals specific differences between the parental inbreds in the character of this effect. The activity of NAD$^+$–GDH in one parent (W-32) increases much more than in the other line (W-187) under the influence of both treatments applied. The increase (percent to the control) of the total activity of NAD$^+$–GDH under the action of Siapton and L-glutamic acid was determined (Figure 2). It was established that the difference between the lines in that increase is 58% (P = 0.1%) for the experiments with L-glutamic acid treatment, and 37% (P = 1%) for the Siapton treatment experiments. Inasmuch as the degree of heterosis manifested by a single-cross hybrid is a function of the genetic difference of its parental inbreds, we used the differences established between the genotypes as additional characteristics besides those obtained in the comprehensive study of the metabolism of inbred lines for the purpose of hybridization in accordance with a preset laboratory program (Mladenova et al., 1978).

Our results suggest that the effect of L-glutamic acid on the deaminating activity of GDH in the leaves of 14-day-old maize plants is not specific. An amino acid mixture obtained after protein hydrolysis produces a similar effect. Moreover, these results show that the glutamate–α-ketoglutarate system is also affected by the slightest concentration changes of the free amino acids. In relatively low concentration this system is known to bring about a transamination of various amino acids with different keto acids as acceptors, whereby the composition of the amino acid pool in the cells and organisms may be qualitatively regulated in accordance with the needs of protein biosynthesis. This regulation could be achieved by regulating the activity of NAD$^+$–GDH, one of the enzymes catalyzing and controlling the above system.

Our data might be examined also with a view to the probable mechanism of action of the foliar organic fertilizers. It is not known what metabolism spheres might be influenced by the exogenous action of "balanced" mixtures of free amino acids (protein hydrolysates). Any amino acid in a great excess might interfere in the delicate mechanism of amino acid synthesis, intracellular transport or protein synthesis, but it is less easy to understand how a slight amino acid excess in a balanced mixture could produce such a strong effect (Joy, 1969).

Our results show that the biological effect of free amino acids in the Siapton organic fertilizer might be connected with their effect on the action of the glutamate–α-ketoglutarate system in the leaf tissue of different maize genotypes.

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