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Nucleotide-dependent isomerization of glutamate dehydrogenase in relation to total RNA contents of peanut

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Abstract

The physiological function of glutamate dehydrogenase (GDH) was investigated by treating germinating peanut (*Arachis hypogaea* L.) seeds with nucleoside triphosphate (NTP) solutions in order to alter the isoenzyme distribution patterns. The free nucleosides and nucleotides of the GTP-treated peanut were the highest [8.7 μmol g^{-1}(f.m.)], and they decreased through the ATP-treated peanut [5.8 μmol g^{-1}(f.m.)], and CTP-treated peanut [5.5 μmol g^{-1}(f.m.)], to the UTP-treated peanut [4.1 μmol g^{-1}(f.m.)]. The combination of 4 NTPs induced 20 % higher content of Pi [173 nmol g^{-1}(f.m.)] than in the control, but the combined ATP+UTP treatment induced the lowest [93.0 nmol g^{-1}(f.m.)] Pi. The 4 NTP treatment also induced the highest number of GDH isoenzymes (28) followed by the purine NTP treatments (15 to 20), but the pyrimidine NTP treatments and the combined purine + pyrimidine NTP treatments induced the lowest numbers (<15) of isoenzymes. The deamination/amination ratios were generally higher in the UTP (0.11), and CTP (0.06) treated peanuts than in the GTP (0.04), and ATP (0.07) treated peanuts. There were mutual relationships between higher numbers of GDH isoenzymes present in the GTP-, and ATP-treated peanuts and higher RNA (236.5 and 239.4 μg g^{-1}, respectively) contents on one hand, and between the lower numbers of isoenzymes in the CTP-, and UTP-treated peanuts and lower RNA (162.0 and 152.5 μg g^{-1}, respectively) contents. The recurrent relationships of the effects of the NTP treatments of peanut were UTP > ATP > CTP > GTP.

Additional key words: *Arachis hypogaea*, chromatography, deamination/amination ratio, free nucleotides, inorganic phosphate.

Introduction

Glutamate dehydrogenase (GDH) (EC 1.4.1.2) is a multi-isoenzymic oxidoreductase. It plays important roles in cellular carbon and nitrogen metabolism (Osuji and Madu 1995, Aubert *et al.* 2001). Its isomerization reaction has been demonstrated both *in vivo* (Nauen and Hartmann 1980, Cammaerts and Jacobs 1983, Srivastava and Singh 1987, Watanabe *et al.* 1992) and *in vitro* (Osuji *et al.* 2001) thus attesting to the chemical basis of the reaction (Osuji *et al.* 1999). The enhancement of biomass following the genetic transformation of plants with the *gdhA* gene (Lightfoot *et al.* 1999, Ameziane *et al.* 2000) shows that the enzyme plays a major role in plant growth. But its biochemical and molecular mechanisms, especially the relationship between the isoenzyme distribution pattern, the oxidative deamination, and reductive amination reactions have remained largely unclear because most of the research approaches either focused attention on the effects of sugar and protein catabolism, or relied on the suppression of the reductive activity in order for the oxidative activity to be monitored. Accordingly, the responses of the enzyme to the changes in nitrogen and carbon metabolism have been reported extensively (Ratajczak *et al.* 1981, Lettgen *et al.* 1989, Zink 1989, Leon *et al.* 1990, Lea *et al.* 1992, Robinson *et al.* 1992, Stewart *et al.* 1995, Osuji and Madu 1997a, Aubert *et al.* 2001) without explanation of the isoenzyme distribution patterns in terms of the oxidative and reductive activities of the enzyme, and in terms of the regulation of carbon and nitrogen assimilation. However, the coordinate regulation of

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Abbreviations: IEF - isoelectric focusing; NTP - ribonucleoside triphosphate; NDP - ribonucleoside diphosphate; NMP - ribonucleoside monophosphate; α-KG - α-ketoglutarate; Pi - inorganic phosphate.

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carbon and nitrogen assimilation pathways is only beginning to be understood (Coruzzi and Bush 2001, Coruzzi and Zhou 2001). Improved understanding of the molecular mechanisms of the enzyme will broaden the scope of its biotechnological applications (Lightfoot *et al.* 1999). But GDH changes its isoenzyme population distribution pattern in response to different concentrations of its inducers (Loulakakis and Roubelakis-Angelakis 1991, Osuji *et al.* 1997/98, Osuji *et al.* 1998) thus suggesting that its oxidative and reductive components may be functionally interdependent, and quantitatively related to the isoenzyme distribution patterns. GDH also isomerizes in response to the nucleophilicity of its inducers (Osuji 1997, Osuji *et al.* 1999, Osuji and Braithwaite 1999), thus suggesting that nucleotides rather than amino acids and NH_4^+ might be the preferred modulators *in vivo*. The responses of the aminating and deaminating activities of the enzyme to changes in the breakdown and interconversion of nucleotides have not been studied. Nucleotides are used for RNA synthesis. They also play important regulatory roles in carbon and nitrogen metabolism (Wasternack 1978, Osuji and Ory 1987, Lollier *et al.* 1995, Senecoff *et al.* 1996), and in signal transduction (Harden *et al.* 2001), their cellular concentrations being regulated by reciprocating feedback mechanisms. Although the pyrimidine and purine rings of nucleotides are synthesized in all cells, the activities of the synthetic pathways are relatively lower than the salvage pathways which retrieve the nucleic acid bases after DNA and RNA degradation (Osuji and Ory 1986, Smith and Atkins 2002).

Therefore, it is important to study the response of GDH to the changes in the degradation and salvage of nucleotides. Since GDH plays important roles in plant growth (Ameziane *et al.* 2000, Osuji *et al.* 2003a), cellular carbon and nitrogen metabolism, it is expected that its *in vivo* molecular mechanisms will be illuminated by a study of the interaction between the GDH isoenzyme patterns and the reciprocating regulation of nucleotide concentrations especially in the background of the nontranscriptional synthesis of RNA by the enzyme *in vitro* (Osuji *et al.* 2003b). The results presented hereunder divert attention from the redox property of GDH because they show that the enzyme isomerized in response to the concentrations of free nucleotides, and that the number of its isoenzymes and their distribution patterns regulated the total RNA contents.

Materials and methods

Germination of seeds: Peanut (*Arachis hypogaea* L. cv. Valencia) seeds were sterilized with *Clorox* for 10 min, washed twice with sterile distilled water, and germinated on filter paper moistened with distilled water, 0.5, 0.2, or 0.1 mM ribonucleoside triphosphate (NTP) solution at ambient temperature (24 to 29 °C), and local daylight conditions during the period of May - June, 1996 and 1997. All solutions were changed at 24 h intervals. Five weeks after germination, when the leaves of the control seedlings were turning yellow, the entire seedlings per treatment were washed with distilled water, briefly dried with tissue paper, and immediately pulverized to powder in liquid nitrogen with mortar and pestle. The powder was stored at -80 °C.

GDH extraction: GDH was extracted (Osuji and Madu 1995) from the powdered seedlings (35 to 40 g) by homogenization at 4 °C with 100 cm^3 of extraction buffer (Loyola-Vargas and De Jimenez 1984), and finally purified by preparative-scale isoelectric focusing (IEF; *Rotofor*, *Bio-Rad*, Hercules, USA) as described before (Osuji and Madu 1995).

GDH isoenzyme pattern: Equal volumes (0.3 cm^3) of the *Rotofor* fractions were concentrated about 8-fold by vacuum centrifugation; followed by electrophoresis at 4 °C through 7.5 % native PAGE (Osuji and Madu 1995). GDH isoenzyme pattern was visualized by staining the electrophoresed gel in L-glutamate-NAD^+-phenazine methosulphate-tetrazolium blue solution (Cammaerts and Jacobs 1983).

GDH assay: GDH activities were determined by *DU-64* spectrophotometer (*Beckman*, Fullerton, USA) at 340 nm (Osuji *et al.* 1997b). For the determination of the amination activity, concentrations of 7.0 mM α-keto-glutarate (α-KG), 50 mM NH_4Cl, 0.16 mM NADH, 1.3 mM $CaCl_2$, and 0.2 cm^3 of rotoforated GDH were used in a total reaction volume of 3.0 cm^3 per assay. For the determination of the amination kinetic constants, 0.4 to 35.0 mM α-KG, 2.0 to 250.0 mM NH_4Cl, 0.16 mM NADH, 1.3 mM $CaCl_2$, and 0.2 cm^3 of rotoforated GDH solution were similarly used. From the initial velocities of the reaction, double reciprocal plots were constructed. The V_{max}, and K_m values were calculated from the replots (Segel 1976) of the 1/V-axis intercepts versus the reciprocals of the NH_4Cl concentrations. All substrates used for the deamination reaction were prepared in 0.1 M Tris-HCl (pH 9.5). Concentrations of 50 mM L-glutamate, 0.6 mM $CaCl_2$, 1.0 mM NAD^+, and 0.2 cm^3 of the rotoforated GDH were used in a total volume of 3.0 cm^3 per assay. GDH assays were done in triplicate with three separate extractions of the enzyme; and their results averaged. Protein contents were determined by the method of Lowry *et al.* (1951).

Isolation of RNA: Total RNA was isolated (Grierson *et al.* 1985) from 10 g of powdered seedlings. RNA

extraction for each treated peanut was done in triplicate. The yield and purity of the RNA were determined by spectrophotometry at 260 and 280 nm. The RNA yields per peanut treatment were then averaged.

Determination of inorganic phosphate: Powdered peanut seedlings (9 g) were homogenized with 30 cm^3 of ice-cold 10 % (m/v) trichloroacetic acid (TCA) in a blendor at maximum speed for 3 min. The homogenate was centrifuged (10 000 *g*, 15 min, 4 °C), and the supernatant was completely recovered. The Pi content of 0.5 cm^3 of the supernatant was determined by the method of Blackwell *et al.* (1990) with the Ames acid molybdate/$FeSO_4$ reagent against a calibration curve prepared using phosphate standards made up in 2.5 % (m/v) TCA.

Determination of free nucleotides: Powdered peanut seedlings (9 g) were homogenized with 30 cm^3 of ice-cold 70 % (v/v) ethanol in a blendor at maximum speed for 3 min. The homogenate was centrifuged (10 000 *g*, 15 min, 4 °C) to remove precipitated proteins and nucleic acids. The supernatant was completely recovered and kept on ice. An aliquot of the supernatant corresponding to the extract from 3 g of the seedlings was vacuum-concentrated to ~1 cm^3, and then chromatographed through a 5-cm^3 cartridge of *Econo-Pac Q* (*Bio-Rad*) strongly basic anion exchanger. After loading the extract into the cartridge, the automated *Econo* System was programmed at a flow rate of 5 cm^3 min^{-1}, and a multi-step of discontinuously increased NaCl concentrations, using distilled water as the initial eluant. The chart recorder speed, and the *Econo* UV monitor wavelength were set at 12 cm per h, and 254 nm, respectively. Elution peaks were collected by time windows. The absorbance at 260 nm of each peak was measured. Nucleotides present in each peak were identified by chromatography of authentic samples. Concentrations were calculated based on the volume of each elution peak, and the molar absorbance at 260 nm (Perbal 1988). Nucleotide extractions were repeated three times and used for the triplicate chromatography of the free nucleotides for each treated peanut. The free nucleotide concentrations were then averaged.

Results and discussion

Seed germination: The seeds treated with distilled water (control), or 0.1 mM NTP solutions recorded 85 to 95 % germination, but those treated with 0.2, or 0.5 mM NTP solutions recorded less than 5 % germination. Therefore, after the germination stage, the seeds treated with 0.2 or 0.5 mM NTP solutions were discarded. The project was then continued with the control and 0.1 mM NTP treatments only. The seedlings grew very well, and in the first 3 weeks, there was no visual difference in growth from one treatment to the other. The seedlings were harvested about 5 weeks after emergence when there was pronounced yellowness in the leaves of the control peanut.

Free nucleotides: The distilled water wash following the sample loading to the *Econo-Pac Q* anion exchanger eluted the nucleosides. Changing the eluant sequentially from distilled water to 0.004, 0.007, and 0.030 M NaCl eluted NADH, NMPs, and NDPs combined with NTPs, respectively (Fig. 1). The free nucleotide elution profile for each treated peanut was very reproducible. Also, all the free nucleotide elution profiles were generally similar because of the presence of the four peaks, but the control peanut was unique due to the presence of ATP peak beside the peak of the NDPs and the other NTPs (Fig. 1*a*). The 0.03 M NaCl eluant therefore eluted two distinct peaks in the control treatment. The nucleotide profiles of the pyrimidine NTP-treated peanuts were very similar, but lacked the purine NMP, and ATP peaks (Fig. 1*b*). The nucleotide profiles of the purine NTP-treated peanuts were also very similar because they contained prominent purine NMP peak (Fig. 1*c*), but lacked the ATP peak. The free nucleotide profiles of the combined purine/ pyrimidine NTP treatments were similar to those of the pyrimidine NTP-treated peanuts in that they lacked the purine NMP, and ATP peaks characteristic of the purine NTP, and control treatments respectively. Therefore the changes in the free nucleotide elution profiles were the evidence that the applied NTPs underwent breakdown followed by interconversion.

Table 1. Concentrations of free nucleosides and nucleotides [μmol g^{-1}(f.m.)] of peanut after treatment with NTPs. Means ± SD.

Eluant	Eluate	Control	ATP	GTP	CTP	UTP	3 NTP	GTP + CTP	ATP + UTP
Water	nucleosides	4.6 ± 0.20	3.7 ± 0.20	5.0 ± 0.30	3.0 ± 0.10	2.8 ± 0.10	4.8 ± 0.30	2.4 ± 0.10	2.0 ± 0.10
0.004 M NaCl	NADH	0.9 ± 0.03	0.7 ± 0.03	0.8 ± 0.03	1.2 ± 0.10	0.5 ± 0.01	0.5 ± 0.01	0.6 ± 0.01	0.5 ± 0.02
0.007 M NaCl	NMPs	0.4 ± 0.01	0.3 ± 0.01	1.1 ± 0.03	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.01	0.1 ± 0.01
0.030 M NaCl	NTPs +	0.5 ± 0.02	0.6 ± 0.01	1.8 ± 0.20	1.2 ± 0.10	0.7 ± 0.01	0.6 ± 0.03	0.6 ± 0.04	0.6 ± 0.05
	NDPs	1.8 ± 0.10							

Although all the nucleotide profiles were generally similar, the nucleotide and nucleoside concentrations were very different (Table 1). The free nucleoside and nucleotide concentrations of peanut (Table 1) were within the range obtained for other biological tissues (Hendersen and Paterson 1973). The nucleoside concentration of each treated-peanut was 20 - 269 % higher than the total nucleotide concentration. The nucleoside and nucleotide concentrations of the GTP-treated peanut were the highest, and they decreased through the ATP, and CTP, to the UTP-treated peanuts. The NMP concentrations of the pyrimidine NTP-treated peanuts were 50 - 80 % lower than those of the purine NTP-treated peanuts. The total concentrations of NDPs, and NTPs were 27 - 283 % higher in the control than in the NTP-treated peanuts. The free nucleotide composition induced by the control treatment was therefore overridden by that induced by the NTP treatment. The changes in the nucleoside and nucleotide concentrations from one peanut to the other were further evidence that the applied NTPs were broken down to nucleosides followed by interconversions to other NMPs, NDPs, and NTPs. The general trend in the effects of the single NTP treatments was that UTP exerted the severest, whilst the GTP exerted the mildest effect.

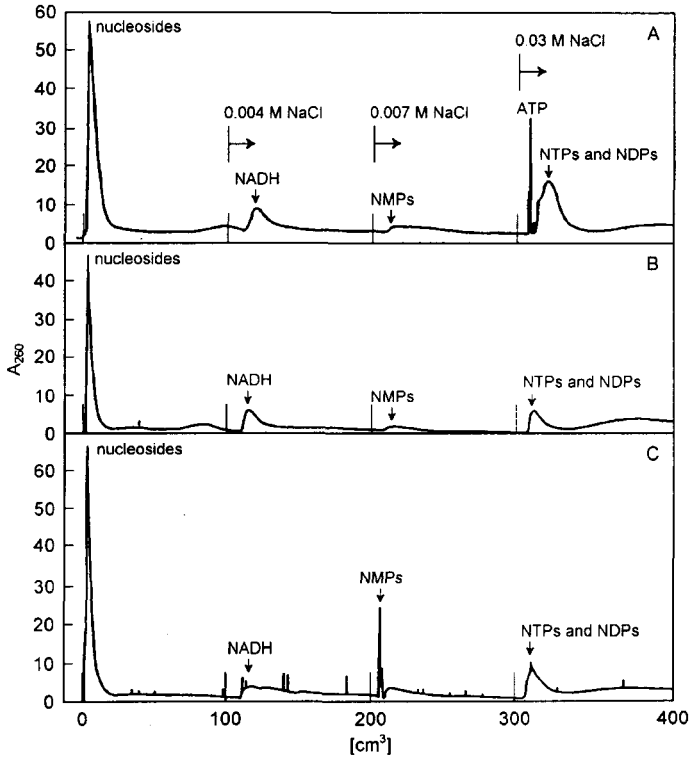

Fig. 1. Free nucleotides of peanut. Frozen peanut seedlings were extracted with 70 % ethanol; the extract was concentrated by freeze-drying, chromatographed on *Econo-Pac Q* strongly basic anion exchanger using increased concentrations of NaCl as the eluant, and the UV absorbance of eluate was measured, with the automated *Econo* System. *A* - control. *B* - UTP-treated peanut, *C* - GTP-treated peanut.

Inorganic phosphate contents: The NTP treatments induced changes in the Pi contents (Table 2). The Pi contents of the peanut were lower than in some other experimental plant tissues (Kakie 1969, Clarkson and Scattergood 1982) probably because inorganic phosphate was not applied to the peanut. The 4 NTP treatment induced the highest Pi level which was about 20 % higher than the control, but the ATP/UTP combined treatment induced the lowest level which was about 34 % lower than the control. Furthermore, the Pi contents decreased from the purine NTP-treated peanuts through the pyrimidine NTP-treated peanuts to the combined purine NTP/pyrimidine NTP-treated peanuts similar to the trends observed in the concentrations of free NMPs, NDPs, and NTPs (Table 1) thus suggesting that the Pi contents were largely derived from the externally supplied NTPs.

Isomerization of GDH in response to NTP treatment: The GDH isoenzyme patterns changed in response to 0.1 mM NTP treatments (Fig. 2). The GDH_1 and GDH_2 nonallelic gene structure (Loulakakis and Roubelakis-Angelakis 1991) with the gene (GDH_1) encoding the more acidic subunits (a), being heterozygous, and codominant; and the gene (GDH_2), encoding the less acidic subunit (b), being homozygous (Cammaerts and Jacobs 1983), explain the isoenzyme patterns in Fig. 2. The binomial distribution of the three types of subunits

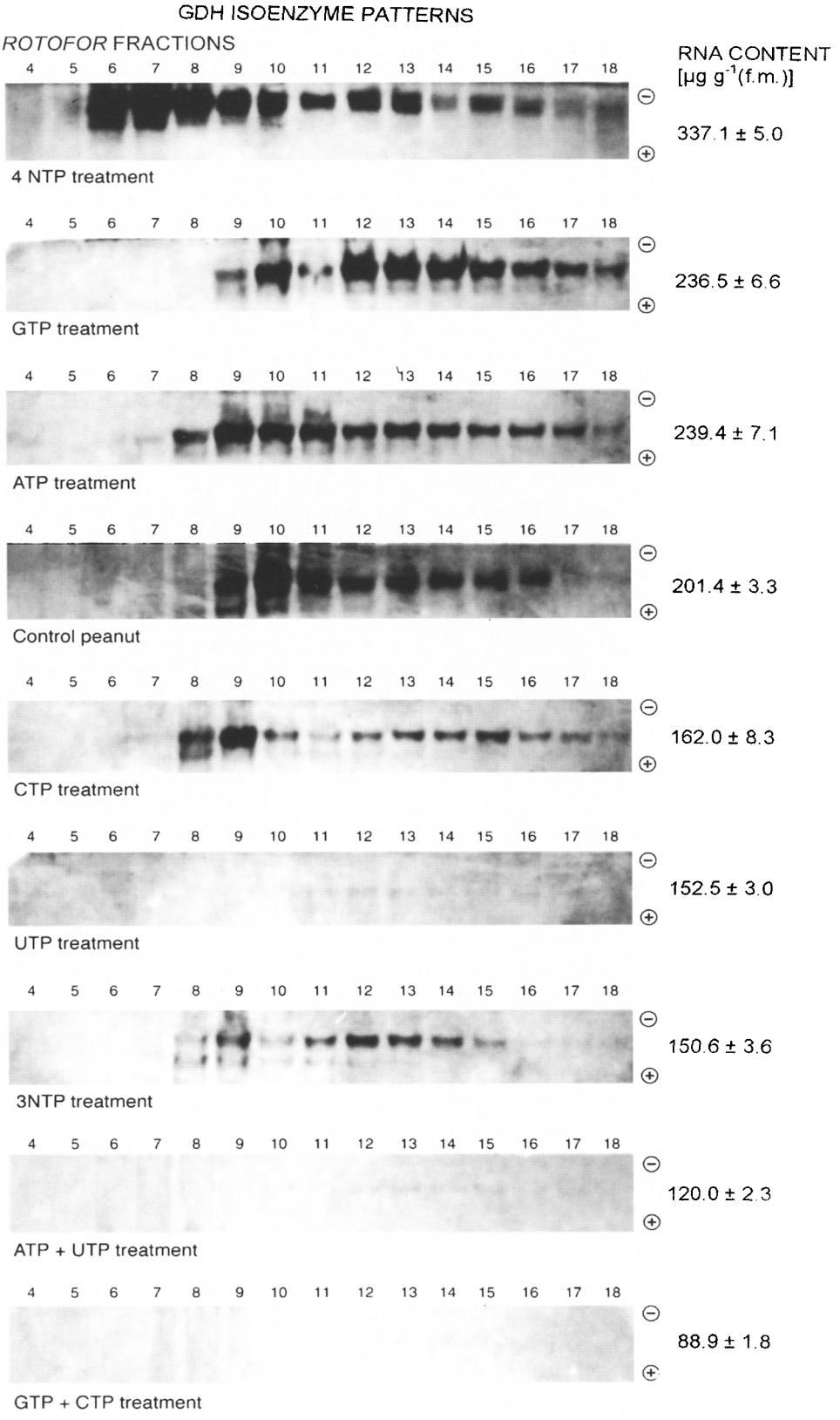

Fig. 2. Effect of different treatments on GDH isoenzyme patterns.

Table 2. Changes in the inorganic phosphate, Pi, contents [nmol g^{-1}(f.m.)] of peanut; GDH amination, GDH deamination, deamination/amination ratio, V_{max} [µmol mg^{-1} min^{-1}], and K_m [mM] of NTP-treated peanut seedlings. Means ± SD.

Treatments	Pi	GDH amination	GDH deamination	Ratio	V_{max}	K_m
Control	143.0 ± 6.0	24.72 ± 1.5	1.44 ± 0.10	0.06	27.8 ± 1.1	8.3 ± 0.3
4NTP	173.0 ± 7.0	16.16 ± 1.1	0.85 ± 0.06	0.05	15.0 ± 0.2	1.6 ± 0.1
ATP	155.0 ± 8.0	10.30 ± 0.8	0.76 ± 0.04	0.07	16.0 ± 1.0	5.4 ± 0.1
GTP	140.0 ± 9.0	14.68 ± 0.9	0.61 ± 0.04	0.04	21.4 ± 0.3	6.9 ± 0.2
CTP	147.0 ± 6.0	16.50 ± 1.0	0.97 ± 0.10	0.06	18.7 ± 1.0	2.7 ± 0.1
UTP	120.0 ± 6.0	15.20 ± 1.2	1.74 ± 0.11	0.11	19.0 ± 0.8	5.0 ± 0.1
3NTP	109.0 ± 8.0	23.81 ± 1.0	1.49 ± 0.10	0.06	25.6 ± 0.2	1.9 ± 0.1
GTP + CTP	127.0 ± 7.0	7.00 ± 0.3	0.73 ± 0.05	0.10	10.2 ± 0.5	1.8 ± 0.1
ATP + UTP	93.0 ± 5.0	10.28 ± 0.7	0.98 ± 0.10	0.10	21.9 ± 0.1	0.8 ± 0.1

accounts for the complex system of 28 hexameric GDH isoenzymes (Osuji and Madu 1995, 1996). Therefore, the *Rotofor* IEF arranged the GDH isoenzymes (Fig. 2) in a gradient from the acidic (oxidative) to the basic (reductive) isoenzymes. The pI values of *Rotofor* fractions 3 - 17 were 4.7, 5.2, 5.5, 5.8, 6.0, 6.3, 6.4, 6.6, 6.9, 7.1, 7.4, 7.6, 7.8, 8.2, and 8.8 respectively. Judging from the numbers of the GDH isoenzyme bands present, the 4 NTP treatment induced the highest number (28), followed by the purine NTP treatments (15 to 20 GDH isoenzymes), while the pyrimidine NTP treatments and the combined purine NTP plus pyrimidine NTP treatments induced the lowest numbers (< 15) of the isoenzymes. The 3 NTP treatments also induced fewer numbers of the isoenzymes than the 4 NTP treatment (Fig. 2). Therefore, the trends in the GDH isoenzyme patterns (Fig. 2) were similar to the trends of the free nucleotide contents (Table 1), and of the Pi contents of the peanuts (Table 2) thus showing that the isomerizations were dependent on the metabolism of the nucleotides. Also, the elution patterns (Fig. 1) of the free nucleotides induced by the treatment with the purine NTPs correlated with the induction of higher numbers of the GDH isoenzymes, whereas the elution patterns of the free nucleotides induced by the treatment with the pyrimidine NTPs correlated with the induction of fewer numbers of the isoenzymes. The effects of the NTPs on the isomerization of GDH (Fig. 2) were different from those of NH_4Cl (Osuji and Madu 1997a), herbicide (Osuji 1997), and pesticides (Osuji and Braithwaite 1999). The results (Figs. 1, 2) therefore showed the existence of close interaction between the GDH isoenzymes and free nucleotides.

Regulation of isoenzyme pattern by oxidation/ reduction ratio: The UTP-treated peanut had the highest oxidative deamination activity of GDH, and it decreased through the 3 NTP, the CTP, and the 4 NTP, to the purine NTP treatments (Table 2). The GDH reductive amination activities followed a similar trend as the deamination activities because the pyrimidine NTP-, 3 NTP-, and 4 NTP-treated peanuts possessed higher amination activities than the purine NTP treatments. The trends of the deamination, and amination activities (Table 2) therefore departed from those of the free nucleotides (Table 2), Pi (Table 2), and isoenzyme distribution patterns (Fig. 2). The GDH amination kinetic constants (Table 2) also followed the trends of the deamination and amination activities. However, taking the GDH deamination and amination together as interdependent functions in the form of oxidation/reduction ratios (Table 2), the trend became similar to those of the isoenzyme distribution pattern, the free nucleotides, and the Pi contents because the oxidation/reduction ratios of the control GDH, the 4 NTP, and the purine NTP treatments were lower than those of the 3 NTP-, and the pyrimidine NTP-treated peanuts. The reciprocal relationship between the deamination and amination activities of GDH was first observed by Leon *et al.* (1990), and it suggested a reciprocal regulation of the physiological function of the enzyme by the cellular oxidation and reduction states. The GDH oxidative deamination activity has for long been regarded as independent of the reductive amination activity (Robinson *et al.* 1990).

Response of RNA contents to GDH isoenzyme patterns: Comparison of the total RNA contents of the peanuts with the respective GDH isoenzyme distribution patterns (Fig. 2) showed that the purine NTP-treated peanuts and the control peanut possessed higher RNA contents than the pyrimidine NTP-treated peanuts. Therefore, the trend of the total RNA contents was in agreement with the trends of the free nucleotides, Pi, and the GDH oxidative/reductive ratio. Although the two elution patterns of the free nucleotides of tissues (Fig. 1) are historical data in biochemistry (Brown 1972), their physiological significance in the regulation of GDH activities remained hitherto unexplained. There were three other trends in the relationship between the RNA contents and the GDH isoenzyme patterns. In the first trend, the RNA contents decreased as the NTP treatment

inactivated the acidic isoenzymes (GDH isoenzymes in *Rotofor* chambers 3 - 10). This was excellently illustrated by the GDH patterns of the 4 NTP-, and the GTP-treated peanuts with GDH isoenzymes focusing only in *Rotofor* chambers 5 - 18, and 9 - 18, respectively, but the 4 NTP-treated peanut contained more RNA [337.1 μg g^{-1}(f.m.)] than the GTP-treated peanut [236.5 μg g^{-1}(f.m.)]. In the second trend, the RNA contents decreased as the nucleotide treatment inactivated the basic isoenzymes (GDH isoenzymes in *Rotofor* chambers 15 - 18). This was illustrated by the GDH patterns of the ATP-, and the 3 NTP-treated peanuts with GDH isoenzymes focusing only in *Rotofor* chambers 8 to 18, and 8 to 15, respectively, but the ATP-treated peanut contained more RNA [239.4 μg g^{-1}(f.m.)] than the 3 NTP-treated peanut [150.6 μg g^{-1}(f.m.)]. The third trend was that as the nucleotide treatments progressively depressed the GDH isoenzymes, the RNA contents of the peanut also decreased. The GTP plus CTP-treated peanut with virtually no visible GDH isoenzyme pattern (Fig. 2) contained the lowest amount of RNA [88.9 μg g^{-1}(f.m.)]. Therefore, the total RNA trends were mutually related to the differential distribution of the GDH isoenzyme population. The corollary is that the GDH isoenzymes imposed a differential distribution on the total RNA species. The RNA polymerase activities of the peanuts were not investigated, but the dependence of the GDH deamination/amination ratio on the free nucleotide concentration, together with the dependence of RNA contents on the differential isoenzyme distribution patterns (Fig. 2) showed that GDH isoenzymes synthesized some RNA *in vivo*. It has already been demonstrated *in vitro*, that the GDH hexameric isoenzymes synthesize RNA independent of DNA and RNA templates (Osuji *et al.* 2003b). Therefore, the synthesis of RNA *in vivo* by GDH isoenzymes is the molecular basis for the regulation of the enzyme by the NTP treatments of the peanut. These results are important for understanding how GDH enhances plant biomass (Ameziane *et al.* 2000, Osuji *et al.* 2003a).

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