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Fractionation of carbon ($^{13}C/^{12}C$) isotopes in glycine decarboxylase reaction

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Abstract Fractionation of carbon isotopes ($^{13}C/^{12}C$) by glycine decarboxylase (GDC) was investigated in mitochondrial preparations isolated from photosynthetic tissues of different plants (*Pisum*, *Medicago*, *Triticum*, *Hordeum*, *Spinacia*, *Brassica*, *Wolffia*). 20 mM glycine was supplied to mitochondria, and the CO_2 formed was absorbed and analyzed for isotopic content. CO_2 evolved by mitochondria of *Pisum* was enriched up to 8‰ in ^{12}C compared to the carboxylic atom of glycine. CO_2 evolved by mitochondria of the other plants investigated was enriched by 5–16‰ in ^{13}C. Carbon isotope effects were sensitive to reaction conditions (pH and the presence of GDC cofactors). Theoretical treatment of the reaction mechanism enabled us to conclude that the value and even the sign of the carbon isotope effect in glycine decarboxylation depend on the contribution of the enzyme-substrate binding step and of the decarboxylation step itself to the overall reaction rate. Therefore, the fractionation of carbon isotopes in GDC reaction was revealed which provides essential isotopic effects in plants in addition to the well-known effect of carbon isotope fractionation by the central photosynthetic enzyme, ribulose-1,5-biphosphate carboxylase.

Key words: Glycine decarboxylase complex; Isotope fractionation; Photorespiration; Plant mitochondria

1. Introduction

Fractionation of carbon isotopes by photosynthetic microorganisms and plants is always connected with the discrimination of ^{13}C in the reaction of enzymatic carboxylation of ribulose-1,5-bisphosphate during photosynthesis [1]. However, there are some data showing that factors affecting photorespiration strongly influence the isotopic content of photosynthetic organisms [2-4]. Analysis of these data led to the suggestion that in the key reaction of the photorespiratory (glycolate) pathway catalyzed by the glycine decarboxylase complex the fractionation of carbon isotopes can take place [5]. The multienzyme glycine decarboxylase complex (GDC) associated with serine hydroxymethyltransferse converts two glycine molecules into one molecule of serine with formation of CO_2, NH_3 and reduction of NAD^+. GDC laeds to the catalytic decarboxylation of glycine and the subsequent transfer of the C_1 fragment to tetrahydrofolate (THF). The selection of stable carbon isotopes in the GDC reaction can result in significant changes in the real isotopic effects during photosynthesis. However, there is no real evidence on the discrimination of carbon isotopes in this reaction.

The goal of the present investigation was to determine the possibility of carbon isotope fractionation in the glycine decarboxylase reaction in vitro. We have anticipated that CO_2 evolved in the reaction should be enriched in ^{12}C compared to the initial substrate as was observed before in the other enzymatic decarboxylation reactions [6-9]. However, we have found that the carbon isotope effect in the above reaction was in most cases of the opposite sign and CO_2 was enriched in ^{13}C. A strong sensitivity of the effect to the reaction conditions was found. The results are discussed below from the standpoint of the reaction mechanism.

2. Materials and methods

For preparation of partially purified glycine decarboxylase complex, mitochondria from green leaves of pea (*Pisum sativum* L.), lucerne (*Medicago sativa* L), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), cabbage (*Brassica napus* L.), spinach (*Spinacia oleracea* L.) and from thalloms of the aquatic plant *Wolffia arrhiza* Hork. were isolated from 100 g of plant material by differential centrifugation [10] and incubated at 25°C in 250 ml Warburg flasks in 50 mM Tris-HCl buffer, pH 8.0, containing 20 mM glycine, 5 mM $MgCl_2$, 8 mM pyridoxal 5-phosphate, 4 mM dithiothreitol, and (if indicated) 2 mM tetrahydrofolate (THF), 8 mM ADP, 2 mM NAD (this facilitates the reaction rate [11]).

In the absence of exogenously added glycine, CO_2 evolution was practically absent (up to 2% of CO_2 evolved in the presence of glycine). Evolved CO_2 was absorbed by 20% KOH in the side arm of the Warburg flask during the initial 2 h of the reaction and then, after the addition of the same amount of glycine, during the following 12 h and converted to the insoluble form ($BaCO_3$). Isolated mitochondria were used because the pure enzyme is rapidly dissociated into separate subunits and it is practically impossible to obtain the pure functional glycine decarboxylase complex [12]. When glycine was added as the sole respiratory substrate to mitochondria, CO_2 could be evolved only in the GDC reaction. Mitochondria actively decarboxylated exogenous glycine during all incubation periods. Practically all CO_2 (about 97–98%) was absorbed by KOH, which was demonstrated in a separate experiment.

The preparation of carbonate samples was carried out using standard techniques [13]. An MI-1201 B mass spectrometer (Russia), with standard deviation of a single measurement of ±0.2‰, was used. The isotopic content of samples is presented in PDB units [14]. Glycine ('Reanal') with a carbon isotopic composition of −25.6‰ was used. In the reaction with ninhydrin this glycine was decarboxylated and the CO_2 derived from its C_1 atoms was analyzed. The isotopic composition of the glycine carboxyl was −26.8‰. All experiments were repeated in threefold and statistically evaluated.

3. Results

In all experiments the isotopic composition of CO_2 evolved during the enzymatic decarboxylation of glycine significantly deviated from that of the substrate (Table 1). In almost all plants investigated (except pea) enrichment in ^{13}C of CO_2 evolved compared to the carbon composition of the substrate was observed. Maximal differences occurred during the first 2 h when up to 10–15% of glycine was converted. In the subsequent 12 h, i.e. when the substrate had been partially (in 30–

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Table 1
Changes in isotopic content of CO_2 carbon evolved during glycine decarboxylation by mitochondria from different plants

Plant	Presence of THF, ADP and NAD	Exposure	
		2 h	Next 12 h
Hordeum	+	15.4	16.0
Triticum	+	5.1	9.4
	–	5.5	3.6
Spinacia	+	16.2	15.2
	–	8.6	3.6
Brassica	+	7.8	4.8
Wolffia	+	7.6	0.1
Medicago	+	10.4	9.9
Pisum	+	0.4	−7.8
	–	−0.6	−1.6

All data are presented in PDB units and expressed in $\Delta^{13}C = \delta^{13}CO_2\text{-}\delta^{13}C_{1(glycine)}$. $\delta^{13}C_{1(glycine)} = -26.8‰$.

40%) depleted, the effect was less evident or even absent (for *Wolffia*). At the end of the experiment alkalinization of the incubation medium by 0.2–0.3 pH units caused by the excretion of ammonium was observed. When the experiment was conducted in the absence of exogenously applied THF, ADP and NAD, there were no significant differences in the first 2 h, but during the following 12 h the effect as compared to the presence of these compounds was lower for most plants. Maximal isotopic effects were observed for spinach, barley ($\Delta^{13}C$=15–16‰), and smaller effects ($\Delta^{13}C$=5–10‰) for wheat, cabbage, and *Wolffia*.

During the decarboxylation of glycine by pea leaf mitochondria in the experiment with addition of THF, NAD and ADP, the CO_2, evolved during the first 2 h was characterized by having an isotopic content similar to that in the substrate ($\Delta^{13}C$=0.4‰). CO_2 collected during the subsequent 12 h was enriched in ^{12}C ($\Delta^{13}C$=−7.8‰). In the other experiment where only pyridoxal 5-phosphate as a cofactor was present, a similar pattern was observed ($\Delta^{13}C$=−0.6–1.6‰), but the isotopic content of CO_2, gathered during the 12 h after first absorption (0–2 h), was much less enriched in ^{12}C than in the first experiment.

4. Discussion

The data obtained show that during the enzymatic decarboxylation of glycine the fractionation of carbon isotopes takes place, which in most cases is connected with the enrichment in ^{13}C of CO_2 carbon, but the reverse effect (in the case of pea) was also observed.

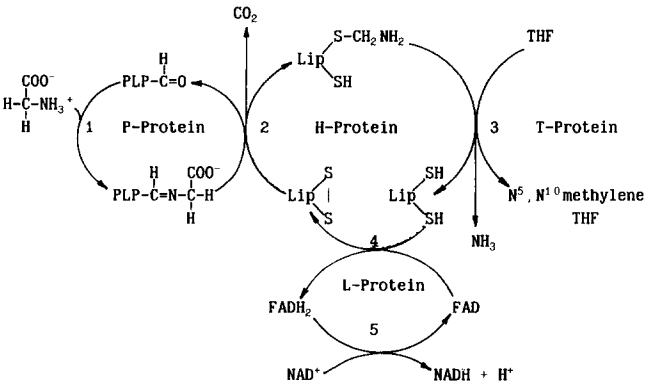

Fig. 1. Reaction model for the glycine decarboxylase multienzyme complex (according to [15]). PLP, pyridoxal 5-phosphate; Lip, lipoamide; THF, tetrahydrofolate.

For an explanation of the data obtained a theoretical treatment of the reaction mechanism is needed. The glycine decarboxylation reaction consists of different steps catalyzed by four types of protein (P, H, T and L) of the glycine decarboxylase complex (see scheme in Fig. 1) [15]. P- and H-proteins in the presence of pyridoxal 5-phosphate and dithiothreitol catalyze the exchange between glycine carboxyl and $^{14}CO_2$ [12]. In the reaction mechanism, the α-amino group of glycine forms a Schiff base with cofactor pyridoxal 5-phosphate in the active site (step 1) and then oxidative decarboxylation of bound glycine takes place (step 2) in which H-protein participates as a cosubstrate. It is evident that only the two first steps catalysed by P- and H-proteins in the presence of pyridoxal 5-phosphate and dithiothreitol can lead to changes in the carbon isotope ratio of CO_2. It is evident that the carbon atom which forms CO_2 takes part directly in chemical transformations only during these initial stages. We can present the formal kinetics of the reaction by a two-stage kinetic scheme

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SE \xrightarrow{k_2} P + E$$

where k_1, k_{-1} and k_2 are the rate constants of the corresponding stages. For this scheme, the carbon isotope effect α_{eff} can be expressed as [16]:

$$\alpha_{eff} = \frac{k_1}{k_1^*} - \frac{1 + k_{-1}^*/k_2}{1 + k_{-1}/k_2} \quad (1)$$

where the asterisk denotes the characteristics of heavy isotopic species. Assuming that $k_{-1} \gg k_2$ and $k_{-1}^* \gg k_2^*$, i.e. there is an equilibrium at the first stage, one can obtain:

$$\alpha_{eff} = \frac{K_1 k_2}{K_1^* k_2^*} = \frac{\alpha_{2kin}}{\alpha_{1therm}} \quad (2)$$

where $K_1 = k_1/k_{-1}$ and $K_1^* = k_1^*/k_{-1}^*$ are the equilibrium constants for the first stage, $\alpha_{1therm} = K_1^*/K_1$ is a thermodynamic isotope effect on the reversible substrate/enzyme binding stage, and $\alpha_{2kin} = k_2/k_2^*$ is a kinetic isotope effect at the irreversible C-C breakage stage.

If $k_{-1} \ll k_2$ and $k_{-1}^* \ll k_2^*$, i.e. equilibrium at the first stage is not achieved, Eq. 1 transforms into Eq. 3:

$$\alpha_{eff} = \alpha_{1kin} = k_1/k_1^* \quad (3)$$

In most reactions of enzymatic decarboxylation the CO_2 evolved is enriched in ^{12}C [6-9]. This means that C-C bond cleavage limits the reaction rate and α_{2kin} is dominant, completely determining α_{eff}, described by Eq. 2. C-C cleavage to a higher degree limits the reaction rate, and the greater part of CO_2 is enriched in ^{12}C as compared with the substrate (its carboxylic carbon).

In our in vitro experiments on glycine decarboxylation, in contrast, CO_2 in most cases was enriched in ^{13}C. This means that the reaction rate was limited by enzyme-substrate binding and described by Eq. 3. CO_2 in this case inherits a carboxylic carbon of enzyme-bound glycine and has the same carbon isotope ratio, since there is no isotope fractionation at the second stage. Within the framework of transition state theory [12], the upper level to which the value of α_{1kin} approaches is an equilibrium isotope effect between enzyme binding (product-like transitional state) and free glycine. The accumulation of ^{13}C in enzyme binding glycine carboxylic group determines the accumulation of ^{13}C in CO_2. Both cases of rate limitation and the corresponding isotope effects of both signs were previously demonstrated for pyruvate decarboxylation [7]. Thus, according to the data obtained for GDC of pea, the decarboxylation rate is limited by the stage of C-C bond breakage, and for GDC of other plants the rate-limiting step is the stage of enzyme-substrate binding. This can be connected with small structural differences in the active site of pea enzyme compared to the enzymes from other investigated plants leading to more rapid enzyme-substrate binding and (or) to the appearance of limitations at the step of CO_2 evolution.

Rate-limiting stages and hence the value and sign of the effect are greatly dependent on reaction conditions. The observed shift of the carbon isotope ratio of CO_2 in the course of the reaction (long-term experiment) seems to be the result of alkalinization of the medium and pH growth due to NH_3 formation at step 3. Isotopic data show that the pH increase may lead to a change in the contribution of rate-limiting steps from step 1 to 2.

The presence of cofactors providing rapid reoxidation of NADH and binding of the methylene group with THF, as follows from the data (see Table 1), also demonstrates a great influence on the carbon isotope effect. NADH competitively inhibits GDC, and the increase in ionic strength and pH leads to dissociation of the complex [17]. The conditions of rapid binding or outflow of the reaction products change the relative contributions of steps 1 and 2.

The data obtained reveal isotopic effects in glycine decarboxylase reaction comparable with the selection of stable carbon isotopes during photosynthetic CO_2 fixation by ribulose-1,5-bisphosphate carboxylase. This provides evidence that operation of GDC in photorespiratory metabolism provides the essential input in redistribution of stable carbon ($^{13}C/^{12}C$) isotopes in photosynthesizing organisms.

References

[1] Smith, B.N. and Epstein, S. (1971) Plant Physiol. 47, 380–384.
[2] Tevsadze, I.T., Tarkhnishvili, G.M. and Sanadze, G.A. (1977) Sov. Plant Physiol. 24, 1073–1075.
[3] Kalinkina, L.G. and Udelnova, T.M. (1990) Sov. Plant Physiol. 37, 96–103.
[4] Kalinkina, L.G. and Udelnova, T.M. (1991) Sov. Plant Physiol. 38, 948–959.
[5] Ivlev, A.A. (1993) Russ. J. Plant Physiol. 40, 872–880.
[6] O'Leary, M.H. (1976) Biochem. Biophys. Res. Commun. 73, 614–618.
[7] Jordan, F., Kuo, D.J. and Monse, E.U. (1978) J. Am. Chem. Soc. 100, 2872–2878.
[8] O'Leary, H.H. and Piazz, G.G. (1981) Biochemistry 20, 2743–2748.
[9] O'Leary, M.H. and Japp, C.J. (1978) Biochem. Biophys. Res. Commun. 80, 155–160.
[10] Lernmark, U., Henricson, D., Wigge, B. and Gardestrom, P. (1991) Physiol. Plant. 82, 338–344.
[11] Douce, R. and Neuburger, M. (1989) Annu. Rev. Plant Physiol. Plant Mol. Biol. 40, 371–414.
[12] Walker, J.L. and Oliver, D.J. (1986) J. Biol. Chem. 261, 2214–2221.
[13] Ivlev, A.A., Kaloshin, A.G., Radyukin, Yu.N., Sholin, A.F. and Pozdnyakova, T.M. (1982) Sov. Microbiol. 51, 194–197.
[14] Craig, H. (1957) Geochim. Cosmochim. Acta 12, 115–149.
[15] Oliver, D.J., Neuburger, M., Bourgignon, J. and Douce, R. (1990) Physiol. Plant. 80, 487–491.
[16] Melander, L. and Saunders, J. (1980) Reaction Rates of Isotopic Molecules, Wiley, New York.
[17] Oliver, D.J. (1994) Annu. Rev. Plant Physiol. Plant Mol. Biol. 45, 323–337.