

# Involvement of cyanide-resistant and rotenone-insensitive pathways of mitochondrial electron transport during oxidation of glycine in higher plants

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**Abstract** Metabolism of glycine in isolated mitochondria and protoplasts was investigated in photosynthetic, etiolated (barley and pea leaves) and fat-storing (maize scutellum) tissues using methods of [ $1\text{-}^{14}\text{C}$ ]glycine incorporation and counting of  $^{14}\text{CO}_2$  evolved, oxymetric measurement of glycine oxidation and rapid fractionation of protoplasts incubated in photorespiratory conditions with consequent determination of ATP/ADP ratios in different cell compartments. The involvement of different paths of electron transport in mitochondria during operation of glycine decarboxylase complex (GDC) was tested in different conditions, using aminoacetonitrile (AAN), the inhibitor of glycine oxidation in mitochondria, rotenone, the inhibitor of Complex I of mitochondrial electron transport, and inhibitors of cytochrome oxidase and alternative oxidase. It was shown that glycine has a preference to other substrates oxidized in mitochondria only in photosynthetic tissue where succinate and malate even stimulated its oxidation. Rotenone had no or small effect on glycine oxidation, whereas the role of cyanide-resistant path increased in the presence of ATP. Glycine oxidation increased ATP/ADP ratio in cytosol of barley protoplasts incubated in the presence of  $\text{CO}_2$ , but not in the  $\text{CO}_2$ -free medium indicating that in conditions of high photorespiratory flux oxidation of NADH formed in the GDC reaction passes via the non-coupled paths. Activity of GDC in fat-storing tissue correlated with the activity of glyoxylate-cycle enzymes, glycine oxidation did not reveal preference to other substrates and the involvement of paths non-connected with proton translocation was not pronounced. It is suggested that the preference of glycine to other substrates oxidized in mitochondria is achieved in photosynthetic tissue by switching to rotenone-insensitive intramitochondrial NADH oxidation and by increasing of alternative oxidase involvement in the presence of glycine.

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**Key words:** Cyanide-resistant oxidase; Glycine decarboxylase complex; Mitochondrial electron transport chain; Photorespiration; Rotenone-resistant NADH dehydrogenase

## 1. Introduction

Interconnection of photorespiratory metabolism and electron transport in mitochondria in glycine oxidative decarboxylation reaction is an important point of regulation of the redox state and energetics of photosynthesizing plant cells [1]. The question arises to what extent respiratory chain par-

ticipates in the oxidation of photorespiratory NADH and to what extent this is coupled with ATP production.

The glycine decarboxylase complex (GDC) is most active in photosynthetic tissues of  $\text{C}_3$  plants and comprises up to one-third of all matrix protein in mitochondria (which provides high photorespiration rates) [2]. Glycine has a preference to other substrates, including succinate, for oxidation in mitochondria and it even suppresses oxidation of exogenous NADH [3]. The lower  $K_m$  of GDC to NAD compared to dehydrogenases of the tricarboxylic acid (TCA)-cycle and active operation of malate-oxaloacetate transporter removing reduced equivalents formed in the GDC reaction from mitochondria were demonstrated [4]. It was shown that oxidation of glycine coupled with NADH oxidation in mitochondrial electron transport chain (ETC) is less inhibited by the inhibitor of Complex I rotenone than oxidation of other mitochondrial substrates [5]. It was also observed that glycine oxidation could continue at appreciable rates even in State 4 conditions [6]. Definite and clear explanation of these results was not presented yet. Nevertheless attempts to explain the preferential oxidation of glycine on the basis of simple kinetic assumptions were unsuccessful for understanding why glycine could suppress oxidation of other substrates when applied in even much less concentration, and how the dehydrogenases of respiratory chain differentiate NADH from different substrates.

Glycine oxidation was suggested to provide the cytosol with ATP [7]. These data were obtained using rapid fractionation of protoplasts incubated in photorespiratory conditions when photosynthetic  $\text{CO}_2$  fixation was operating at 10–50% of maximal  $\text{CO}_2$  fixation. In the conditions of ‘no  $\text{CO}_2$ ’, i.e. when protoplasts are incubated in the  $\text{CO}_2$ -free medium, Rubisco will maintain mainly the oxygenase reaction, and photorespiration will be a very dominating process. This results in significant over-reduction of chloroplast in high light intensities [8]. In these conditions mitochondria can oxidize the excess of photosynthetically formed substrates in ‘overflow’ manner, limiting the over-reduction of the chloroplast redox carriers. Investigation of the participation of different paths of electron transport in these strong photorespiratory conditions was not carried out yet.

In etiolated tissues GDC activity is lower as compared to green tissue [9]. In fat-storing tissue (scutellum) of germinating maize seeds the glyoxylate cycle is coupled with aminotransferase reactions leading to the intensive formation of amino acids [10]. In this tissue glyoxylate is present in significant quantities, and its amination may provide large pools of glycine. In the present investigation we studied the effect of inhibitors on paths of mitochondrial electron transport during glycine oxidation in photosynthesizing tissue and compared

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that with etiolated and fat-storing tissues of plants. Special attention was paid to cyanide and rotenone-insensitive pathways of electron flow and these are suggested to be very important in photosynthetic tissue.

## 2. Materials and methods

Mitochondria were isolated from green leaves of 10-day-old pea (*Pisum sativum* L.) plants, from scutella of 5-day-old maize (*Zea mays* L.) seedlings and from green and etiolated leaves of 8 day old barley (*Hordeum vulgare* L.) plants in self-generated Percoll gradients as described earlier [11]. Oxymetric experiments were carried out at 25°C using a Hansatech oxygen electrode (Norfolk, UK) in: 0.25 M sucrose, 0.25 M sorbitol, 10 mM KCl, 0.5 mM MgCl<sub>2</sub>, 0.06% BSA, 0.2% PVP, 10 mM HEPES–KOH (pH 7.2). The oxygen concentration in the medium was adjusted to air-level (250 µM O<sub>2</sub>) by bubbling with air.

Respiratory control was  $1.6 \pm 0.2$  for mitochondria from fat-storing tissues and  $1.8 \pm 0.2$  for mitochondria from leaf tissues when glycine or malate were applied. There was no essential differences for respiratory control values with glycine or malate.

Exogenous [1-<sup>14</sup>C]glycine (Sigma) was applied at a concentration of 2 mM (radioactivity 0.1 MBq ml<sup>-1</sup>). <sup>14</sup>CO<sub>2</sub> evolved was absorbed during 30 min by monoethanolamine and its radioactivity was measured by liquid scintillation counter [12]. The inhibitors were supplied to mitochondria in the following concentrations: GDC inhibitor aminoacetonitrile, 10 mM; cytochrome oxidase inhibitor KCN, 0.1 mM; Complex III inhibitor antimycin A, 1 µM; alternative oxidase inhibitor salicylhydroxamic acid (SHAM), 1 mM; Complex I inhibitor rotenone, 20 µM. Succinate and malate were applied in a concentration of 5 mM, ADP and ATP concentrations were used in concentrations 0.15 and 3 mM.

Protoplasts were isolated from 8-day-old barley leaves and added to a final concentration of 40 mg Chl ml<sup>-1</sup>. Fractionation experiments were performed using the apparatus of rapid fractionation based on the principle of membrane filtration developed by Lilley et al. [13]. Protoplasts were incubated in 8 ml of assay medium (0.25 M sucrose, 0.25 M sorbitol, 10 mM KCl, 0.5 mM MgCl<sub>2</sub>, 0.06% BSA, 0.2% PVP, 10 mM HEPES, pH 7.2) and illuminated at 500 µmol quanta m<sup>-2</sup> s<sup>-1</sup> with Xenophot HLX slide projector lamps (Osram, Germany) for 5 min for obtaining conditions of steady-state photosynthesis (tested oxymetrically) after the pre-incubation in the dark during 7 min. The rate of photosynthesis was measured in assay medium with 10 mM NaHCO<sub>3</sub> concentration. The light intensity used was saturating for photosynthesis. The protoplasts were first illuminated prior to transfer of the glass syringes to the fractionation equipment. This transfer was made in the light 1 min before fractionation. For providing moderate photorespiratory conditions at 'low CO<sub>2</sub>' (50% of maximal CO<sub>2</sub> fixation) 0.2 mM NaHCO<sub>3</sub> was added to the medium. For strong photorespiratory conditions CO<sub>2</sub>-free assay medium was prepared. Inhibitors were pre-incubated with protoplasts in darkness at room temperature for 4–7 min prior to illumination.

Protoplasts were ruptured on a selected 15 µm nylon net (Henry Simon Ltd., Stockport, UK). Chloroplasts and mitochondria were collected on 8 µm and 0.45 µm membrane filters (MF-Millipore), respectively. The dilution of the samples after rapid fractionation was determined by the comparison of the refractive index of the samples to the refractive index of pure solutions.

Marker enzymes used were: fumarase for mitochondria [14], phosphoenolpyruvate carboxylase for the cytosol [15], and NADP-glyceraldehyde-phosphate dehydrogenase for chloroplasts [16]. Cross-contamination between fractions did not exceed 8%. Isocitrate lyase and malate synthase were determined by the method of Dixon and Kornberg [17]. Chlorophyll concentration was measured according to Bruinsma [18]. ATP was determined by the firefly luciferase method [7]. ADP was converted to ATP by pyruvate kinase (Serva).

## 3. Results

### 3.1. Oxymetric measurement of glycine oxidation

Oxymetric experiments were carried out with mitochondria isolated from green and etiolated barley leaves and from green

Table 1

Effect of inhibitors on glycine oxidation in pea leaf (green) and barley leaf (green and etiolated) mitochondria

Variant	Barley etiolated	Barley green	Pea green
Control	38 ± 4	102 ± 6	101 ± 7
Antimycin	9 ± 1	66 ± 3	72 ± 6
SHAM	30 ± 3	55 ± 4	51 ± 7
Antimycin+SHAM	3 ± 1	12 ± 5	12 ± 6
Rotenone	18 ± 3	84 ± 7	79 ± 8

Rates are nmol O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> mitochondrial protein. Each value represents a mean of 3–4 experiments ± SD.

leaves of pea in State 3 conditions (presence of 0.15 mM ADP) (Table 1). In mitochondria from photosynthetic tissue antimycin A inhibited only by 30% whereas SHAM inhibition showed stronger effect (about 50%). The joint effect of SHAM and antimycin (or cyanide) was about 85–90% in all oxymetric and decarboxylating experiments. Rotenone revealed only about 20% inhibitory effect. In mitochondria from etiolated barley leaves (etiolated pea leaves were not available in enough quantities) the suppression by antimycin was very strong (only 24% of initial activity remained present), whereas SHAM inhibited O<sub>2</sub> consumption only slightly. Rotenone revealed strong inhibitory effect.

Absolute rates of glycine oxidation in mitochondria of green leaves were at the same level as the rates of malate and succinate oxidation even when glycine was applied at saturating concentrations (10 mM). The data for pea leaf mitochondria are given in Table 2. In mitochondria of etiolated leaves and maize scutellum the rates for glycine were 2–3 times lower than the rates for malate and succinate (data not shown).

### 3.2. Decarboxylation of glycine by pea leaf mitochondria

Investigation of GDC activity in intact mitochondria of pea leaves supported the data on preferential oxidation of glycine in mitochondrial electron transport chain (compared to succinate and malate) (Table 3). Low concentration of glycine (2 mM) was used for preventing 'overflow' conditions of glycine supply for mitochondria (*K<sub>m</sub>* of GDC for glycine is 6 mM [4]). Nevertheless there was no visible changes in the involvement of different paths of electron transport when the concentration of glycine increased up to 5 or even 10 mM (data not shown). The addition of succinate led to 1.4-fold stimulation of glycine decarboxylation, the addition of malate to 1.1-fold stimulation. GDC operation in green pea leaves was connected with rotenone-insensitive and cyanide-resistant paths of electron transport (44% inhibition by SHAM and 55% by KCN, almost complete insensitivity to rotenone). Glycine decarboxylation revealed no dependence on its concentration (compared 0.15 and 3 mM) (Table 4). The addition of ATP only slightly suppressed GDC reaction in mitochondria of green pea leaves).

### 3.3. Glycine decarboxylation by mitochondria from maize scutellum

Mitochondria isolated from scutella actively decarboxylated exogenous [1-<sup>14</sup>C]glycine indicating the presence of GDC. Its activity was low in dry seeds, increased 7-fold during 5 days of germination and then declined (Fig. 1). Its pattern corresponds to the dynamics of the glyoxylate-cycle enzymes isocitrate lyase and malate synthase. The maximal GDC activity

Table 2

O<sub>2</sub> consumption by pea leaf mitochondria with different substrates in State 3

Substrate	O <sub>2</sub> uptake
Glycine (10 mM)	101 ± 7
Glycine (2 mM)	73 ± 12
Malate (10 mM)	105 ± 11
Malate (10 mM)+glutamate (10 mM)	112 ± 6
Succinate (10 mM)	97 ± 10

Rates are nmol O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> mitochondrial protein. Each value represents a mean of 4 experiments ± SD.

in scutella was about 4 times lower than in pea leaves, but it comprised similar values as in green leaves of maize and other C<sub>4</sub>-plants [19]. Aminoacetonitrile strongly (65–70%) inhibited oxidative decarboxylation of glycine (Table 3). In the presence of succinate and malate glycine oxidation was strongly (50–60%) suppressed. Incubation of mitochondria with 0.15 mM ATP compared to 0.15 ADP revealed only slight (≈15%) inhibition of decarboxylation rate (Table 5). Glycine conversion was connected both with cyanide-sensitive and cyanide-resistant paths of mitochondrial electron transport (30% inhibition by SHAM in the presence of ADP). Addition of ATP even lead to the increase to cyanide sensitivity. Rotenone revealed 40% inhibitory effect in the presence of ADP and had no significant effect when ATP was added.

### 3.4. Fractionation experiments on barley protoplasts

Protoplasts were incubated in steady-state photosynthesis in 'strong' photorespiratory conditions (absence of photosynthetic O<sub>2</sub> evolution ('no CO<sub>2</sub>')) and in 'moderate' photorespiratory conditions (50% of photosynthetic O<sub>2</sub> evolution (0.2 mM NaHCO<sub>3</sub>, 'low CO<sub>2</sub>')). Rotenone had no effect on chloroplastic and cytosolic ATP/ADP ratios (Fig. 2). In mitochondria it decreased this ratio 2-fold in the conditions of 'no CO<sub>2</sub>' and 'low CO<sub>2</sub>'. AAN lowered ATP/ADP ratio in the cytosol and mitochondria only in the conditions of low CO<sub>2</sub>, but not in the absence of photosynthetic O<sub>2</sub> evolution.

## 4. Discussion

The obtained data indicate that glycine oxidation in mitochondria of photosynthetic tissues is coupled mostly with cyanide-resistant and rotenone-resistant paths of electron trans-

port contrary to etiolated leaves and fat-storing tissue where the preference of glycine to other mitochondrial substrates was not observed and cyanide-sensitive path was involved in much less extent. Cyanide inhibition of GDC itself is expressed in some extent, but less than that of serine hydroxymethyltransferase [20], and really glycine oxidation may be even less cyanide sensitive than observed in experiments (we do not consider this inhibition).

If the mitochondrial matrix has a uniform NADH pool, it is difficult to explain how the dehydrogenases of the respiratory chain differentiate NADH from different substrates [8]. However, oxidation of glycine could inhibit oxidation of other NAD-dependent substrates by the maintenance of high NADH/NAD<sup>+</sup> ratio in mitochondrial matrix as GDC is present in green leaf mitochondria in high concentration and its affinity to NAD<sup>+</sup> (*K<sub>m</sub>* 75 μM) is higher than that of the TCA-cycle enzymes malate dehydrogenase and isocitrate dehydrogenase (100–250 μM) [4].

The unique preference of glycine to other mitochondrial substrates in photosynthesizing plant cell cannot be explained on the basis of simple kinetic assumptions by high content of GDC and its high affinity to NAD<sup>+</sup>. We used non-saturating concentration of glycine in order to maintain its flow not at the maximal rate whereas malate and succinate were used in concentration 5 mM which is significantly higher than *K<sub>m</sub>* of corresponding dehydrogenases. Even at saturating concentrations (10 mM) glycine oxidation by mitochondria from green leaves proceeded with the rates at the same level as the rates of malate and succinate, but its preference to these substrates was always observed. The obtained data indicate that special molecular mechanisms exist providing preferential oxidation of glycine in photosynthetic tissue. Synergistic effect of operation of GDC with malate dehydrogenase and succinate dehydrogenase may provide explanation for stimulation of glycine oxidation in the presence of malate and succinate. Preliminary data of existence of metabolons of GDC with malate dehydrogenase were presented earlier [21]. Succinate oxidation in the presence of glycine is suppressed strongly [22], as well as malate oxidation [5].

The enhancement of glycine decarboxylation in the presence of malate and succinate is an important aspect of provision of the preference of glycine oxidation compared to other substrates. Previously [23] it was shown that CO<sub>2</sub> and NH<sub>3</sub> release from glycine in State 3 is not inhibited in the presence of malate and succinate. Even the enhancement of CO<sub>2</sub> release has been even observed. Malate may act as a source of pyruvate (via malic enzyme reaction) which is a specific activator

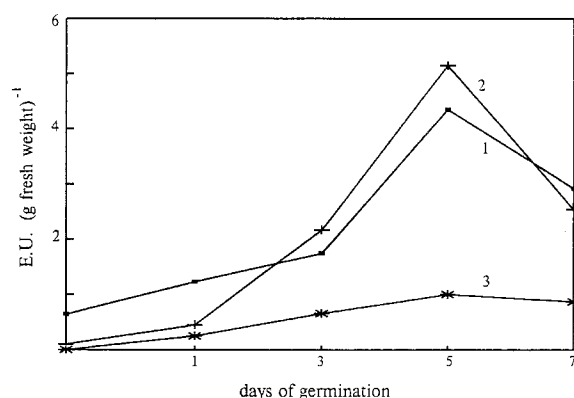


Fig. 1. Changes of activities of glycine decarboxylase Complex (1), isocitrate lyase (2) and malate synthase (3) in maize scutella during germination of seeds.

Table 3

Decarboxylation of [1-<sup>14</sup>C]glycine by mitochondria of green pea leaves and of maize scutellum in the presence of inhibitors and metabolites

Variant	nmol O <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> mitochondrial protein	
	pea leaf	maize scutellum
Control	181 ± 18	39 ± 4
+KCN	81 ± 21	22 ± 3
+SHAM	102 ± 14	28 ± 4
+KCN+SHAM	26 ± 13	8 ± 3
+Rotenone	170 ± 24	44 ± 6
+Succinate	253 ± 35	14 ± 3
+Malate	212 ± 26	18 ± 3
+AAN	46 ± 6	14 ± 2

Each value represents a mean of 3–4 experiments ± SD.

Table 4  
Decarboxylation of [1-<sup>14</sup>C]glycine by mitochondria of green pea leaves in the presence of different ADP and ATP concentrations

Variant	nmol CO <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> mitochondrial protein			
	0.15 mM ADP	3 mM ADP	0.15 mM ATP	3 mM ATP
Control	177 ± 21	169 ± 27	154 ± 23	150 ± 32
+KCN	114 ± 27	74 ± 34	110 ± 43	126 ± 21
+SHAM	118 ± 16	103 ± 27	67 ± 18	110 ± 25
+Rotenone	190 ± 35	157 ± 19	116 ± 29	129 ± 13

Each value represents a mean of 4 experiments ± SD.

of the reduced form of alternative oxidase [24]. The activation of alternative path may be also provided by succinate and malate themselves [25] but in an indirect way via stimulation of pyruvate formation [26]. Malate and other organic acids of the TCA cycle also stimulate operation of malate-oxaloacetate shuttle transferring redox equivalents from mitochondria to cytosol [27]. Thus, in oxidation of reduced equivalents formed in GDC reaction the alternative path of mitochondrial electron transport may be increasingly involved which provide regulation of energetic state of mitochondria and prevent their over-reduction.

In mitochondria from photosynthetic tissues of C<sub>3</sub>-plants ATP and ADP did not significantly influence the control of glycine oxidation which is expressed in changes of involvements of different paths of electron transport, linked (in State 3) or non-linked with oxidative phosphorylation. Even at high ATP concentration glycine is metabolized intensively and electron transport from NADH formed in glycine decarboxylase reaction is suppressed insignificantly. Investigation of glycine oxidative decarboxylation in the presence of ATP and ADP shows that adenylate control on glycine oxidation is very small. Glycine oxidation practically is not suppressed in the presence of ATP, and only redistribution of electron fluxes via paths connected and non-connected with ATP generation takes place in photosynthetic tissues. Experiments with rotenone show that NADH formed during glycine oxidation avoids the Complex I and its oxidation is realized via alternative NADH dehydrogenase non-connected with generation of proton gradient and ATP synthesis.

GDC in fat-storing tissue (maize scutellum) can utilize glycine formed from glyoxylate which production is significant during operation of the glyoxylate cycle. The pattern of development of the GDC activity similar to the activity of the glyoxylate-cycle enzymes isocitrate lyase and malate synthase reveals correspondence between glyoxylate-cycle operation and utilization of glycine. GDC therefore plays a role in amino acid conversion during seed germination. Glycine in mitochondria of maize scutellum, contrary to mitochondria of photosynthetic tissues, had no preference to other mitochondrial substrates supplying redox equivalents to electron transport chain. It reveals strong rotenone-resistance when ATP was added, but cyanide insensitivity even decreased in the presence of ATP (compared to the total level of glycine oxidation in that tissue), therefore ATP does not participate significantly in redistribution of paths between cytochrome oxidase and alternative oxidase. The presence of other than in green tissue form of alternative oxidase non-sensitive to pyruvate and reducing agent (dithiothreitol) [28] can provide explanation for these results. Therefore in etiolated and fat-storing tissues glycine oxidation has no such peculiarities as in photosynthetic tissue, although small sensitivity to transfer-

ring of mitochondria to ATP surplus is evident, and it at least partially is explained by the involvement of rotenone-resistant path.

Experiments on rapid fractionation of protoplasts reveal that in the conditions of steady-state photosynthesis at high light intensities the role of mitochondria may consist mainly in the prevention of the 'over-reduction' of the plant cell. Results indicate that in steady-state photosynthesis the role of rotenone-resistant pathway becomes more important, especially for glycine oxidation in photorespiratory conditions. When CO<sub>2</sub> is depleted in the medium, this oxidation becomes non-coupled with ATP production possibly passing via alternative oxidase. Therefore strong photorespiratory conditions, in which glycine flux is prevailing in mitochondria, lead to the absence of supply of cytosol by ATP, contrary to moderate photorespiratory conditions in which CO<sub>2</sub> fixation is present. In strong photorespiratory conditions ATP/ADP ratio is very high and the paths of ETC non-coupled with ATP are most active. The involvement of alternative oxidase may be provided by the mechanism of its covalent activation by reduction of its disulfide bond under high level of reduced equivalents in mitochondria (this is connected with maintaining of high NADH/NAD<sup>+</sup> ratio during GDC operation because of lower K<sub>m</sub> of GDC to NAD<sup>+</sup> than that of the TCA-cycle enzymes [4]. The other aspect of its regulation is connected with pyruvate accumulation, as NH<sub>4</sub><sup>+</sup> formed in the GDC reaction activates kinase of pyruvate dehydrogenase leading to inactivation of pyruvate dehydrogenase complex [29].

Pyruvate acts as activator of alternative oxidase. Regulation of rotenone-insensitive bypass is more obscure as NAD(P)H dehydrogenases participating in this path has not been characterized yet. We can suggest mechanism providing the association of rotenone-insensitive internal NADH-dehydrogenase with glycine decarboxylase or its direct activation by glycine or products of its conversion. Association of the TCA-cycle dehydrogenases with Complex I was shown [30], so possible association of GDC with malate dehydrogenase [21] may interfere with this association.

The involvement of paths non-coupled with ATP synthesis in strong photorespiratory conditions may provide the defence

Table 5  
Decarboxylation of [1-<sup>14</sup>C]glycine by mitochondria of maize scutellum in the presence of 0.15 mM ADP and 0.15 mM ATP

Variant	nmol CO <sub>2</sub> min <sup>-1</sup> mg <sup>-1</sup> mitochondrial protein	
	0.15 mM ADP	0.15 mM ATP
Control	43 ± 6	40 ± 3
+KCN	39 ± 4	23 ± 2
+SHAM	19 ± 2	27 ± 4
+Rotenone	25 ± 4	43 ± 5

Each value represents a mean of 3 experiments ± SD.

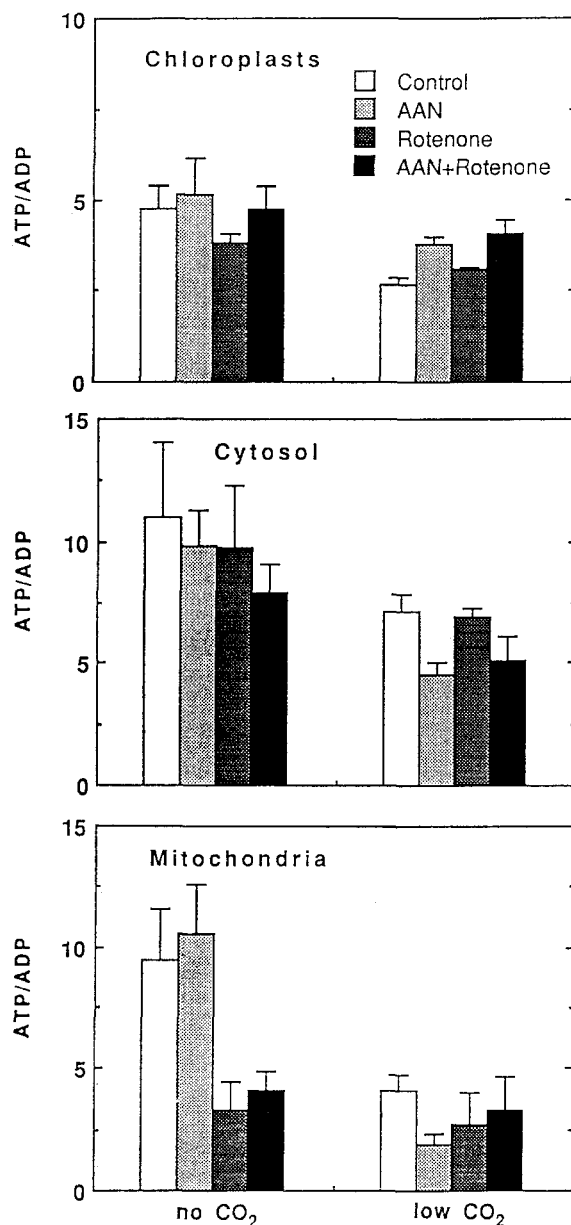


Fig. 2. ATP/ADP ratios in chloroplasts, cytosol and mitochondria rapidly fractionated from barley protoplasts incubated in the light in conditions of the absence of photosynthetic CO<sub>2</sub> evolution (no CO<sub>2</sub>) and of the 50% of saturating CO<sub>2</sub> evolution (low CO<sub>2</sub>) in the presence of aminoacetoneitrile (AAN) and rotenone.

from formation of active oxygen forms which are intensively produced when ubiquinone pool is in a reduced state [31]. Mechanisms providing connection of glycine oxidation with paths non-coupled with ATP synthesis may be important in the prevention of over-reduction in mitochondria and therefore of oxygen damage.

In mitochondrial matrix glycine oxidation has influence on ATP/ADP ratio even in strong photorespiratory conditions ('no CO<sub>2</sub>'). Therefore in these conditions cytochrome path during glycine oxidation may be connected with supply of ATP only for mitochondrial matrix, not cytosol. How this is achieved via suppression of ATP transfer across mitochondrial membrane, is a problem for future investigation. It may be connected with electrochemical potential values of inner mitochondrial membrane.

The results obtained show that mitochondria of photosynthetic tissues acquired the mechanisms of involvement of cyanide-resistant and rotenone-insensitive pathways during glycine oxidation in higher extent than during oxidation of other substrates which plays a role in redox regulation during photosynthetic metabolism and in ensuring of the continuous operation of photorespiratory cycle. In non-green tissues GDC participates in the process of amino acid conversion which is active in fat-storing tissue during operation of glyoxylate cycle. In these tissues glycine has no preference to other mitochondrial substrates.

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