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The function of glycine decarboxylase complex is optimized to maintain high photorespiratory flux via buffering of its reaction products

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ABSTRACT

Oxidation of glycine in photorespiratory pathway is the major flux through mitochondria of C3 plants in the light. It sustains increased intramitochondrial concentrations of NADH and NADPH, which are required to engage the internal rotenone-insensitive NAD(P)H dehydrogenases and the alternative oxidase. We discuss here possible mechanisms of high photorespiratory flux maintenance in mitochondria and suggest that it is fulfilled under conditions where the concentrations of glycine decarboxylase reaction products NADH and CO_2 achieve an equilibrium provided by malate dehydrogenase and carbonic anhydrase, respectively. This results in the removal of these products from the glycine decarboxylase multienzyme active sites and in the maintenance of their concentrations at levels sufficiently low to prevent substrate inhibition of the reaction.

1. Introduction

Photorespiration is one of major metabolic processes associated with photosynthesis. By consuming oxygen and releasing CO_2 it has participated in equilibrating these gases in the atmosphere for hundreds of millions of years. Photorespiration functions to recycle 2-phosphoglycolate produced as a consequence of the oxygenase reaction of Rubisco (Bauwe et al., 2010; Maurino and Peterhänsel, 2010). During its operation, it prevents overreduction of photosynthetic cells (Igamberdiev et al., 2001; Osmond et al., 1997) and depletion of CO_2 from the active sites of the key enzyme of photosynthetic metabolism (Roussel and Igamberdiev, 2011; Roussel et al., 2007). Different cell compartments (chloroplasts, peroxisomes, mitochondria and cytosol) host particular reactions of the photorespiratory process, while the key reaction of two glycine molecules being converted to one serine molecule is localized in mitochondria and catalyzed by glycine decarboxylase complex (GDC) and serine hydroxymethyltransferase (SHMT). GDC is the most abundant soluble protein in green leaf mitochondria of C3 plants (Douce et al., 1994) and, as we will discuss in the following, it shows the characteristic functional regulation related to the maintenance of high enzyme activity levels to support metabolic needs of photorespiratory carbon flow.

The optimal operation of Rubisco in photosynthesizing cells requires a continuous rapid delivery of CO_2 to its active site because the concentration of Rubisco in the chloroplast stroma exceeds the concentration of its substrate CO_2 by 2–3 orders of magnitude (Igamberdiev and Roussel, 2012). This delivery is achieved through the action of carbonic anhydrases present in the chloroplast stroma and thylakoids (Park et al., 1999). The reaction of Rubisco also requires alternative kinetic approaches to describe photosynthetic CO_2 assimilation (Igamberdiev and Roussel, 2012). The issues regarding the kinetics of Rubisco are not unique to this enzyme. Indeed, several enzymes (which we can call *engine enzymes*) are present at high concentrations relative to the concentrations of the corresponding substrates (Harris and Königer, 1997). This condition will generally result in the depletion of substrate in the proximity of the active sites of enzymes. In these cases, a great deal of the substrate will be tied up in enzyme–substrate complexes which will tend to favor metabolite channeling, i.e. direct passing of the product of one enzyme-catalyzed reaction to the next enzyme in a conversion pathway. This ensures that once a substrate enters a pathway, it is converted to the ultimate product of the pathway with high probability (Easterby, 1989). In simple terms, a huge buildup of enzyme concentration to increase metabolic flux makes sense only in cases where there is a mechanism of continuous pumping of the substrate to the active site. In such case, as long as the influx of substrate is equaled by the capacity of its enzymatic conversion, the conditions will be optimal for the enzymatic reaction. This pumping mechanism

Abbreviations: CA, carbonic anhydrase; DPI, diphenyleneiodonium; ETC, electron transport chain; GDC, glycine decarboxylase complex; MDH, malate dehydrogenase; NDA, rotenone-insensitive, DPI-insensitive, Ca^{2+}-independent internal NADH dehydrogenase; NDB, rotenone-insensitive, DPI-sensitive, Ca^{2+}-dependent external NADH and NADPH dehydrogenases; NDC, rotenone-insensitive, DPI-sensitive, Ca^{2+}-dependent internal NADPH dehydrogenase; OAA, oxaloacetate; SHMT, serine hydroxymethyltransferase.

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includes operation of corresponding *buffering enzymes*, such as carbonic anhydrase for Rubisco (Igamberdiev and Roussel, 2012) and adenylate kinase for ATP synthase (Igamberdiev and Kleczkowski, 2003). The main requirement for the buffering enzymes is that they should operate several orders of magnitude faster than the engine enzymes to provide fast turnover of ligands in the proximity of the engine enzymes' active sites.

In this paper, we analyze the operation of another important plant enzymatic complex GDC that in C3 plants can reach up to 50% of the total protein concentration in mitochondria from green tissues (Douce et al., 1994). The impairment in the GDC even by 30–50% leads to the accumulation of glycine, increased susceptibility to drought and to conversion of glyoxylate to formate (Bykova et al., 2005; Heineke et al., 2001; Wingler et al., 1999a,b), which indicates that the physiologically adequate rate of glycine oxidation is close to its maximal rate at the present ambient air CO_2 concentration of ~400 ppm. This is explained by high concentrations of glycine generated in photorespiring tissues approaching 25 mM in the cytosolic fraction of barley leaves (Winter et al., 1993). Consequently, high GDC and SHMT concentrations are required for providing maximum capacity photorespiratory flux through mitochondria that can reach up to a third of photosynthetic flux rate, and it is essential that the enzyme operates near maximum capability. There are four glycine-binding sites per GDC complex, which has a molecular weight of 1.3 MDa (Oliver et al., 1990), thus there is a binding site per 325 kDa. The matrix concentration of GDC approaches 0.13 g ml^{-1} (Oliver et al., 1990) or even, according to some estimations, 0.3 g ml^{-1} (Douce et al., 2001), which alters the density of mitochondria. If we assume that there is 400 mg protein per ml in the matrix, and we further assume that 40% of it is GDC, then we can calculate that the concentration of glycine-binding sites of the P-protein of GDC is near 0.5 mM, which is comparable to the concentration of Rubisco in chloroplasts (Pickersgill, 1986). The rates of glycine oxidation in isolated mitochondria are usually in the range of 200 nmol (O_2 consumed) mg^{-1} (mitochondrial protein) min^{-1} while the rates of isocitrate oxidation (a "bottleneck" in the TCA cycle) are no more than 50 nmol mg^{-1} min^{-1} (Day and Wiskich, 1977). In the presence of malate the rate of glycine oxidation in isolated mitochondria increases to 400 and higher nmol (O_2 consumed) mg^{-1} (mitochondrial protein) min^{-1} (Bykova and Møller, 2001; Bykova et al., 1998; Wiskich et al., 1990), which is close to the possible maximum photorespiratory flux in vivo. This rate corresponds to the catalytic constant of GDC on the order of ten per second (10 s^{-1}) that is comparable to the rate of Rubisco (3 s^{-1}) and it is below the value of catalytic constant of malate dehydrogenase (MDH) (1000–3000 s^{-1}) by two–three orders of magnitude (Walk and Hock, 1976). The catalytic constant was reported to be 6.8 s^{-1} for the P protein of GDC from chicken (Fujiwara and Motokawa, 1983).

While the concentration of the GDC substrates (glycine and NAD^+) may exceed the concentration of GDC subunit proteins (Igamberdiev et al., 2001; Winter et al., 1993), its products (NADH, CO_2 and possibly NH_3) can strongly inhibit the complex when they accumulate in close proximity of the GDC active sites and are not efficiently removed (via buffering by the corresponding enzymes). While the efficient operation of Rubisco is supported by a strong influx of CO_2 into the chloroplast stroma provided by fast equilibration of bicarbonate and CO_2 and forwarding the latter to Rubisco reaction centers through the action of carbonic anhydrase, the efficient operation of GDC can be supported by the dynamic equilibrium of NADH-NAD^+ redox state by MDH and the CO_2-bicarbonate equilibrium actively maintained by the mitochondrial carbonic anhydrase.

2. Formation of NADH and the buffering role of malate dehydrogenase

Glycine oxidation (as well as decarboxylation) in plant mitochondria is strongly facilitated in the presence of malate (Bergman and Ericson, 1983; Wiskich and Dry, 1985). It was proposed that oxaloacetate (OAA) formation during malate oxidation may, in the reverse MDH reaction, recycle NADH formed in the GDC reaction and in the forward MDH reaction facilitate NADH oxidation in the respiratory chain of mitochondria (Wiskich and Dry, 1985; Wiskich et al., 1990). In fact, the addition of both malate and OAA significantly stimulated the rate of glycine oxidation with stronger effect of OAA (Day et al., 1985a; Woo and Osmond, 1976). The addition of glutamate in the presence of malate, which results in transamination of OAA and its removal via aspartate aminotransferase, results in similar stimulation of glycine oxidation as malate alone (Wiskich et al., 1990). The observed stimulation by malate in the presence of glutamate is even more pronounced and stable (N.V. Bykova, unpublished). The hypothesis of Wiskich et al. (1990) assumes the existence of separate metabolons of MDH, one serving for oxidation of photorespiratory NADH via OAA reduction and another participating in the oxidation of malate in the TCA cycle (Wiskich et al., 1990). However, there has been no experimental verification of separate MDH pools in mitochondria. Indeed, metabolon organization of the electron transport chain (ETC) respiratory complexes into respirasomes of different composition (Dudkina et al., 2006; Krause et al., 2004) and possibly of the TCA cycle enzymes (Sweetlove and Fernie, 2013; Vélot et al., 1997) has been confirmed but there is no evidence for enzyme clustering or metabolon formation in relation to the oxidation of photorespiratory glycine.

Based on the recently described strategy for understanding the operation of enzymes with low turnover rates but present at high concentrations (Igamberdiev and Roussel, 2012), we can postulate that there is no need for separate metabolic domains in order to facilitate the removal of NADH by MDH from the proximity of GDC. The main condition is a higher turnover rate and activity of the buffering enzyme MDH, where there is insufficient free GDC molecules present (with no NADH in the active site) to perform new reactions. In the case of high concentration of NADH-producing enzymes, MDH can successfully contribute to the equilibrium of NADH/NAD^+ in mitochondria (the catalytic constant in the direction of OAA reduction is more than 10^3 s^{-1} as compared to the catalytic constant of GDC (less than 10 s^{-1}). Thus, the affinity of GDC for NADH will significantly decrease when MDH is simultaneously operating in agreement with the kinetic properties of enzymes present at high concentrations (Hanson and Schnell, 2008). In accordance with this kinetics, the value of Ki becomes inflated in the presence of MDH due to the equilibration and thus removal of NADH generated by GDC from its proximity. Mitochondrial MDH operates close to the state of equilibrium (Hagedorn et al., 2004; MacDougall and apRees, 1990), which means that low NADH/NAD^+ ratio is supported by this very active enzyme. This will result in the attenuation of GDC inhibition by NADH (reported Ki 15 μM) (Oliver et al., 1990). The NADH/NAD^+ ratio tends to increase during active respiration and photorespiration (Igamberdiev et al., 2001), and the equilibrium of MDH (malate to OAA ratio ~50 at pH 7.5) (Cuevas and Podestá, 2000) will push it to low values. It is important in this connection that the Km (NADH) value of MDH (Cuevas and Podestá, 2000) is similar to the Ki (NADH) value of GDC.

The reactions taking place in mitochondria oxidizing glycine in the presence of malate and glutamate are shown on Fig. 1. The average activities and kinetic constants of participating enzymes are presented in Table 1. The buffering role of the mitochondrial MDH was thoroughly analyzed in the study by Hagedorn et al. (2004), where it is calculated that for the operation of TCA cycle, 1/10 of the MDH activity in mitochondria would be sufficient. The rest of activity supports the buffering role of MDH. Most of the OAA produced, besides the condensation with acetyl-CoA, can be transaminated with glutamate due to a high activity of aspartate aminotransferase in plant mitochondria (Wilkie and Warren, 1998). However, this will not significantly displace the MDH equilibrium because of a lower k_{cat} (OAA) of the plant mitochondrial aspartate aminotransferase (~300 s^{-1}) and its similar (or higher) Km for OAA (38–56 μM) (Wilkie and Warren, 1998), as compared to Km for