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Role of organic acids in the integration of cellular redox metabolism and mediation of redox signalling in photosynthetic tissues of higher plants

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ABSTRACT

Organic acids play a crucial role in numerous metabolic processes accompanied by transfer of electrons and protons and linked to the reduction/oxidation of major redox couples in plant cells, such as NAD, NADP, glutathione, and ascorbate. Fluxes through the pathways metabolizing organic acids modulate redox states in cell compartments, contribute to generation of reactive oxygen and nitrogen species, and mediate signal transduction processes. Organic acid metabolism not only functions to equilibrate the redox potential in plant cells but also to transfer redox equivalents between cell compartments supporting various metabolic processes. The most important role in this transfer belongs to different forms of malate dehydrogenase interconverting malate and oxaloacetate or forming pyruvate (malic enzymes). During photosynthesis malate serves as a major form of transfer of redox equivalents from chloroplasts to the cytosol and other compartments via the malate valve. On the other hand, mitochondria, via alterations of their redox potential, become a source of citrate that can be transported to the cytosol and support biosynthesis of amino acids. Citrate is also an important retrograde signalling compound that regulates transcription of several genes including those encoding the alternative oxidase. The alternative oxidase, which is activated by increased redox potential and by pyruvate, is, in turn, important for the maintenance of redox potential in mitochondria. The roles of organic acids in establishing redox equilibrium, supporting ionic gradients on membranes, acidification of the extracellular medium, and regulation of production of reactive oxygen and nitrogen species are discussed.

1. Introduction

Energy in living organisms is generated through the energized electrons of reduced compounds, transiently stored in proton gradients and accumulated in macroergic phosphate bonds. The fluxes of protons and electrons are separated and reunited within a complex network that constitutes the basis of cellular metabolism [1]. The energy of proton gradients is accumulated in ATP and other macroergic compounds, while the energy of electrons is stored in the pairs of redox compounds, the most important of which are NADPH/NADP$^+$ (mainly driving anabolism), NADH/NAD$^+$ (mainly driving catabolism) as well as the additional redox pools (ascorbate/dehydroascorbate, reduced/oxidized glutathione). In addition to these redox pairs, an important role in the storage and transfer of electrons and protons belongs to organic acids, which are particularly important in plant metabolism. Organic acids play a major role in the transformation of redox energy into the energy of macroergic bonds via the energy of proton gradients. Their oxidation supplies electrons to the mitochondrial electron transport chain while their stored pools can accumulate redox energy for prolonged times and spend it when needed. These pools can also easily transfer electrons and protons via membranes, since the large coenzymes, such as NADH and NADPH, do not cross most membrane systems. In metabolism, the role of organic acids consists in buffering redox states and transferring redox equivalents to other compartments. They can be considered as a mobile form of redox energy with the redox pairs (malate/OAA, isocitrate/2-oxoglutarate) that contribute to kinetic flexibility of cellular metabolism.

The existence of separate NAD and NADP pools in living cells determines their participation in the catabolic and anabolic processes respectively. Availability of NAD in the oxidized state facilitates the oxidative reactions, while NADP is present in a more reduced state promoting the reactions of reductive biosynthesis [2]. Plants usually have much lower NADPH/NADP$^+$ ratio than animals due to the absence of proton-translocating transhydrogenase [3], and also a lower

Abbreviations: GDC, glycine decarboxylase; ICDH, isocitrate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; OAA, oxaloacetate; 2-OG, 2-oxoglutarate; OGDC, 2-oxoglutarate dehydrogenase complex; Trx, thioredoxin; PDC, pyruvate decarboxylase complex; RNS, reactive nitrogen species; ROS, reactive oxygen species

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ATP/ADP ratio due to the absence of creatine kinase [4], resulting in the animal cells being much more energized. Although animal cells transiently accumulate organic acids in the processes of incomplete oxidations, such as lactate formation during glycolysis, they do not store organic acids at the scale of plant cells. Organic acids in plants constitute the transient pools of fixed carbon accumulated due to altering transient conversion times of intermediates in metabolic cycles and pathways. Their transient nature means that they can either be converted back to carbohydrates or undergo terminal oxidation yielding CO_2 and H_2O [5]. In the present paper we will emphasize the role of organic acids in regulation of metabolism and redox signalling. In particular, we will discuss redox buffering role of organic acids and their participation in the regulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) production and scavenging. We will review the role of di- and tricarboxylic acids such as malate and citrate in metabolism and redox signalling. While the role of other acids, in particular of the ascorbate-dehydroascorbate redox pair, is especially important in coordination of the chloroplast and mitochondrial metabolism in the light [6–9], their role is extensively reviewed in other papers [10,11].

2. Malate dehydrogenase equilibrium

2.1. Malic acid and malate dehydrogenase equilibrium

Malic acid plays a central role in plant metabolism [12] mediated by active malate dehydrogenase isozymes which are present in almost all cell compartments. In many plants, malate is the most accumulated acid fulfilling the roles of an osmolyte and an anion, and compensating the positive charge of potassium and other anions, e.g., during stomatal responses [13]. Malate is considered the main substrate of plant respiration [14,15], such that the TCA cycle can operate on malate as a sole carbon source replenishing intermediates of the cycle provided that both malate dehydrogenase (forming OAA) and malic enzyme (forming pyruvate) are operating. During light-enhanced dark-respiration (LEDR) malate becomes the major or even sole source for operation of the TCA cycle [16].

Malate is converted by NAD- and NADP-dependent malate dehydrogenases (MDH; EC 1.1.1.37 and EC 1.1.1.82) and by NAD- and NADP-dependent decarboxylating malate dehydrogenases called malic enzymes (ME; EC 1.1.1.39 and EC 1.1.1.40). While NADP-malate dehydrogenase is present only in chloroplasts, NAD-malate dehydrogenases exist in numerous molecular forms being very active in different cell compartments. A high activity of NAD-MDH results in conditions where the substrates and products, i.e. NAD^+, NADH, OAA and malate, are effectively equilibrated. The equilibrium constant of MDH ([OAA][NADH]/[Malate][NAD^+]) is in the order of $\sim 3 \times 10^{-5}$ [17], tending to decrease with an increase of pH, resulting in the establishment of malate/OAA ratios of > 400 at pH 6.8 and ~ 50 at pH 7.5 [18]. Since the reaction equilibrium is strongly displaced towards malate and NAD^+, the catabolic redox charge (NADH/(NADH + NAD^+)) and NADH/NAD^+ ratio, exhibit quite low values.

The ratio NADH/NAD^+ in the cytosol of photosynthetic cells is 10^{-3} [19]. The ratio is higher in mitochondria (10^{-2}–10^{-1}) and in chloroplasts (0.3–0.4), increasing in plants with impaired photorespiration [20]. This may indicate that NAD-MDH has insufficient capacity in chloroplasts to equilibrate NADH/NAD^+ due to its low activity [21], while in mitochondria NAD-MDH operates close to its equilibrium under conditions where substrate turnover within the TCA cycle results in the transient accumulation of NADH [22]. Malate stimulates oxidation of important respiratory and photorespiratory metabolites that are converted by large multienzyme complexes. This effect can be related to the establishment of an MDH equilibrium favouring operation of corresponding multienzyme complexes (see Sections 2.2 and 2.3). NAD-MDH involvement in the balance of NADH and NAD^+ during oxidation of mitochondrial substrates is schematically presented on Fig. 1.

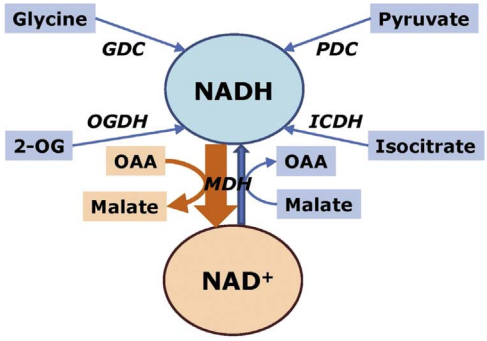

Fig. 1. The role of NAD-malate dehydrogenase in the equilibration of NAD^+ and NADH. The NADH generated during oxidation of mitochondrial substrates is pushed to low level due to the equilibrium of NAD-malate dehydrogenase which is displaced toward formation of NAD^+ and malate. The establishment of low NADH/NAD^+ ratio via the equilibrium of NAD-malate dehydrogenase is particularly important during the intensive production of NADH in photorespiratory oxidation of glycine, in pyruvate oxidation, it may also facilitate oxidation of other substrates.

Being important in the redox control in different cell compartments, NAD-MDH itself is not regulated directly by the redox potential (e.g. via thioredoxin) but exerts control by adenine nucleotides, decreasing its activity with the increase of the ATP/ADP ratio, which was demonstrated for the mitochondrial form [23]. Its two subunits can be regulated by phosphorylation [24]. NAD-malic enzyme is another mitochondrial protein which is phosphorylated [24].

2.2. The role of malate dehydrogenase in maintaining NADH/NAD^+ equilibrium during glycine oxidation

Glycine turnover due to its oxidative decarboxylation represents the most intensive flux in photorespiring mitochondria exceeding the rate of respiration, which is suppressed in the light [25]. Its rate in isolated pea mitochondria is in the range of 200 nmol (O_2 consumed) mg^{-1} (mitochondrial protein) min^{-1}, considerably greater than the rate of isocitrate oxidation at less than 50 nmol mg^{-1} min^{-1} [26]. Upon addition of malate to mitochondria the rate of glycine oxidation increases to more than 400 nmol (O_2 consumed) mg^{-1} (mitochondrial protein) min^{-1} [27–29], which provides an estimate of the maximum photorespiratory flux in vivo. This rate is achieved by the glycine decarboxylase complex (GDC) having the catalytic constant of 5–10 s^{-1} while the value of catalytic constant of NAD-MDH is by two–three orders of magnitude higher (1000–3000 s^{-1}) [30,31].

The protein concentration of GDC in the mitochondrial matrix is in the range of 0.1–0.3 g ml^{-1} [32,33], increasing the matrix density of mitochondria in photosynthetic tissues. This corresponds to the concentration of glycine-binding sites of the P-protein of GDC of ~ 0.5 mM [33]. A high flux of glycine oxidation involves non-coupled pathways of mitochondrial NADH oxidation [34]. GDC operates below substrate saturation allowing flexible regulation of the photorespiratory flux in response to environmental changes [35,36]. In the course of photorespiration, the GDC substrates glycine and NAD^+ are present at higher concentrations than the GDC subunit proteins [21,37]. However, the products of GDC reaction NADH, CO_2 and possibly NH_3 can strongly suppress operation of the complex when they are not efficiently removed. The mechanisms of removal of CO_2 and possibly NH_3 can include participation of the mitochondrial carbonic anhydrase, while the removal of NADH can be achieved via the dynamic equilibrium of NADH-NAD^+ redox state [25].

Early observations with plant mitochondria revealed that glycine oxidation/decarboxylation is strongly facilitated in the presence of malate [38,39]. This stimulation was explained by recycling of NADH in the reaction with OAA in the reverse MDH reaction, which is confirmed by even higher efficiency of OAA to stimulate GDC [28,39].