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Review

Citrate and isocitrate in plant metabolism

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Abstract

The relevance of citrate and isocitrate metabolism in plants is discussed in connection with the different pathways for their conversions. The routes for citrate and isocitrate conversions are incorporated into the system of cross-linked metabolic processes and may provide carbon skeletons for nitrogen assimilation and reducing equivalents for biosynthetic reactions, support the functioning of the glyoxylate cycle and play an important role in the TCA and energy metabolism as a whole. The possibility of the coupling of citrate and isocitrate metabolism with various electron transport systems is discussed from the point of view of the efficiency of the balancing cellular NAD(P)H/NAD(P)$^+$ and ATP/ADP ratios. The role of citrate and isocitrate and their derivations as potent effectors of some enzymes is considered. Special attention is paid to the enzymes associated with citrate and isocitrate metabolism and to the mechanisms which regulate their activity. The possibilities of the coordination of the main processes of energy and biosynthetic metabolism at the level of citrate and isocitrate distribution are discussed.

Keywords: Plant metabolism; Citrate; Isocitrate; Enzyme; Regulation mechanism

Contents

Abbreviations: CS, citrate synthase (EC.4.1.3.7); TCA, Tricarboxylic acid cycle; AOX, alternative oxidase; AH, aconitate hydratase (EC.4.2.1.3); NAD$^+$–ICDH, NAD$^+$-specific isocitrate dehydrogenase (EC.1.1.1.41); NADP$^+$–ICDH, NADP$^+$-specific isocitrate dehydrogenase (EC.1.1.1.42); ATP–CL, ATP–citrate lyase (EC.4.1.3.8); ICL, isocitrate lyase (EC.4.1.3.1); GS, glutamine synthetase (EC.6.3.1.2); GOGAT, glutamate synthase (EC.1.4.1.14); GDH, glutamate dehydrogenase (EC.1.4.1.2); PDH, pyruvate dehydrogenase (EC.2.7.1.99); GDH, glutamate decarboxylase (EC.4.1.1.15); SHAM, salicylhydroxamic acid; PK, pyruvate kinase (EC.2.7.1.40); A–CoAH, acetyl–CoA hydrolase (EC.3.1.2.1); A–CoAC, acetyl–CoA carboxylase (EC.6.4.1.2) PFK, phosphofructokinase (EC.2.7.1.11); PDC, pyruvate dehydrogenase complex; GABA, γ-aminobutirate; ETC, Electron transport chain

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1. Introduction

The interaction and coupling of various metabolic pathways ensures the unified and regulated system of metabolic flows both in the cell and in the whole plant. A key role in this system belongs to the regulation of assimilatory and energy metabolism under cell conditions. Cellular metabolism can be characterised by the existence of pathway branchpoints, where the coordinated distribution of metabolite flows between different processes occur. From our point of view, the enzymatic conversions of citrate and isocitrate can play the role of such branchpoints in plant metabolism. Citrate and isocitrate belong to the key metabolites in plant cells. Their conversions are mediated by several enzymes of central metabolic pathways located in different cellular compartments and involve both assimilatory and energy metabolism [1–3]. The paths for citrate and isocitrate conversions are incorporated into the system of cross-linked metabolic processes and are controlled by numerous factors. The processes related directly or indirectly to the TCA and energy production, nitrogen metabolism, fatty acid synthesis, the glyoxylate cycle and photorespiration are among the most important of these factors. In the present review, the significance of citrate and isocitrate is discussed in connection with their branchpoint position in plant cellular metabolism. Some aspects of the regulation of citrate and isocitrate conversing enzymes are considered, especially with respect to the control of the major metabolic pathways involved in both biosynthetic and energy metabolism.

2. Enzymes and metabolites involved in citrate and isocitrate conversions

Citrate belongs to the compounds which plant tissues usually contain in considerable amounts. Currants, wild strawberries, lemons, spinach and haricot leaves contain from 8% to 15% citrate, based on dry weight [4]. The oxalacetate condensation with the acetyl–CoA, catalysed by CS, is the basic way of citrate synthesis. During this condensation, the acetyl group of acetyl–CoA adds to the si-face of keto moiety of oxaloacetate under inversion of the configuration of the methyl group. The citryl–CoA is an intermediate in the reaction [5] (Fig. 1).

The citrate metabolisation in plant cells can be carried out by several pathways. The basic way of citrate conversion is its oxidation via the reactions of the TCA. In this case, citrate conversion into isocitrate is catalysed by AH, and *cis*-aconitate is formed as an intermediate product. Some data suggest the existence of aconitate isomerase in plants, which catalyses the isomerization of *cis*-aconitate into *trans*-aconitate [6]. There are indications that some plants are able to accumulate *trans*-aconitate in considerable amounts [4,7].

Some plants, like *Kalanchoe* leaves are unable to accumulate large amounts of isocitrate, mostly in their succulent parts [8]. ICDH catalyses the oxidative decarboxylation of isocitrate to 2-oxoglutarate. There are two forms ICDH, NAD^+–ICDH and $NADP^+$–ICDH, both highly specific to their coenzymes [1,3]. Enzyme-bound oxalosuccinate forms as an intermediate during catalysis by $NADP^+$–ICDH. However, we

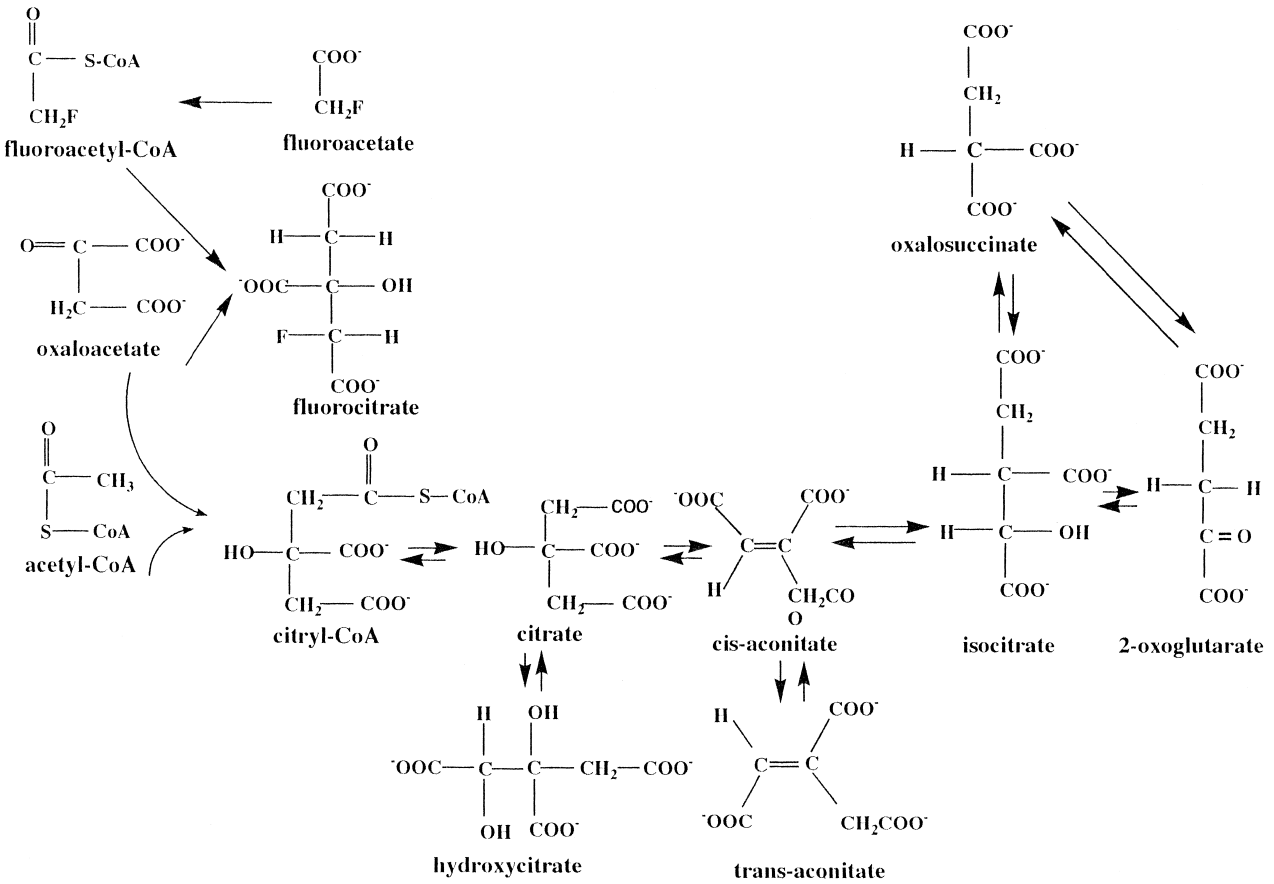

Fig. 1. Metabolites linked with citrate and isocitrate conversions in a plant cell.

no have evidence in favour of the formation of oxalosuccinate during catalysis by NAD^+–ICDH [3]. There are some indications that the catalysis by NAD^+–ICDH is not reversible [9,10]. In contrast, the $NADP^+$–ICDH-catalysed reaction is easily reversible [11]. This may be connected with different enzyme functions in isocitrate metabolism.

Citrate can act not only as a metabolite of the reactions associated with the TCA, but also as an important acetyl donor for acetyl–CoA synthesis by ATP–CL. ATP–CL catalyses the conversion of citrate and CoA to oxaloacetate and acetyl CoA.

Besides this, citrate can be metabolised via the glyoxylate cycle reactions in the plant tissues, with the pathway being actively functional. In this case, isocitrate formed by the AH is further split into glyoxylate and succinate under the action of glyoxysomal ICL. However, ICL reaction is reversible and its synthase function can play a role for isocitrate formation through glyoxylate condensation with succinate [12,13]. It has been suggested that this isocitrate is decarboxylated by cytosolic $NADP^+$–ICDH, yielding 2-oxoglutarate, which in turn produces glutamate. The presence of this metabolic route, which can be considered as an alternative route of photorespiratory glyoxylate metabolism in green leaves, was shown using labelled succinate [14,15] (see below).

Plants also can contain such forms of citrate as fluorinecitrate and hydroxycitrate. Fluorinecitrate is formed from flourineacetate and accumulated by some plants, e.g., *Diehapetalum cytosum* and *Acida georgina* [16,7]. Hydroxycitrate was found in *Hibiscus sabdariff* flowers [17] and *Adinandra braseii* wood [18]. Hydroxycitrate formation can be connected with 4-hydroxy-2-oxoglutarate conversion or with direct condensation of glyoxylate and malate.

Different pathways of citrate and isocitrate conversions are shown in Fig. 1.

There is evidence of multiple forms of citrate and isocitrate conversing enzymes in plants. These molecular forms participate in several metabolic pathways and have specific regulatory properties, that can promote the coordination of metabolic flows through a competition for the common cellular citrate and isocitrate pool. For example, different forms of CS localised in mitochondria and glyoxysomes were discovered in higher plants [19–21]. In some early papers several forms of AH were found in cytosol, glyoxysomes and mitochondria [7] However, it should be noted that recent articles have shown the absence of AH in the glyoxysomes [22–24]. NAD^+– and $NADP^+$–ICDH, showing marked differences in their catalytic properties and physiological roles, have been found in several plant sources [1,3]. NAD^+–ICDH is localised uniquely in mitochondria, while $NADP^+$–ICDH has been found in different compartments such as the cytosol, mitochondria, chloroplasts and peroxisomes [25–30]. ICL is mostly localised in glyoxysomes. However, it was shown that extraglyoxysomal forms of ICL are present in plant tissues which accumulate no fats in cytosol and in mitochondrial fraction [31,12,13]. It has been found that the cytosolic form of the enzyme has some kinetic and regulatory properties which differ strongly from those of the glyoxysomal form [12]. There are some indications that the existence of different isoforms of ICDH [25,32,26,33,34], ICL [35] and AH [7] is genetically determined.

Thus, we can conclude, that citrate and isocitrate conversions proceed in different cellular compartments and are mediated by various enzymatic isoforms characterised by the particular catalytic parameters. The whole complex of enzymatic activities ensures the existence of a highly organised branchpoint of cellular metabolism. Some metabolites linked with this branchpoint can be transported through the organelle membranes by the special systems, thus providing close interrelation of the intracellular processes in different compartments [36–38]. Intermediates and enzymes of the citrate and isocitrate conversions are present in a cell in various forms capable of fulfilling particular functions and often playing a regulatory role in some metabolic processes, due to the specific peculiarities of their structure (see below).

3. Citrate and isocitrate conversions as the branchpoint of metabolic pathways in plants

The interrelation between the main metabolic pathways and citrate or isocitrate conversions is a characteristic of cellular metabolism organization.

The citrate and isocitrate conversions are the initial stages of the TCA. The reactions catalysed by CS and NAD^+–ICDH may be the crucial points of the TCA rate regulation [19,39,1,3,5]. It is known that the TCA is an important source of redox equivalents for oxidative phosphorylation. However, the TCA together with its anapleurotic reactions works not only as a source of reducing equivalents for the electron transfer chain (ETC), but also as a source of intermediates for lipogenesis, organic and amino acid synthesis. It is thus reasonable to consider it as a central point of intracellular metabolism as a whole. The existence of two types of ICDHs appears to reflect a specific feature of the functioning of the cycle in catabolism and anabolism concurrently. The TCA rate is believed to depend significantly on the activity of NAD^+–ICDH, while the $NADP^+$–ICDH participates in biosynthesis [1,3].

The product of the $NADP^+$–ICDH reaction, NADPH is employed in various biosynthetic processes. Cytosolic NADPH could come from the pentose phosphate pathway [40]. But, the cytosolic $NADP^+$–ICDH might be an alternative source for generating at least part of the NADPH needed for the processes of reductive biosynthesis in the cytosol. There are some indications that the $NADP^+$–ICDH reaction is the metabolic stage which contributes significantly to the generation of NADPH, with the level of NADPH being in some cases not only comparable, but even exceeding that of NADPH formed in the pentose phosphate pathway [41–43]. Some indications suggest that the pentose phosphate pathway can provide only one-fifth to one-third of the NADPH required for lipid biosynthesis in developing oil-rich seeds [44].

Another role played by $NADP^+$–ICDH is connected with the supply of carbon skeletons for NH_4^+ assimilation into an organic skeleton via chloroplastical GS and GOGAT [1,45,46,3]. GS, GOGAT and, to some extent, GDH are believed to have dominant roles in the biosynthesis of glutamate and glutamine [46,47]. In all these enzyme systems, the ICDH reac-

tion product, 2-oxoglutarate, is the main nitrogen acceptor and serves as precursor of glutamic acid, a central metabolite in nitrogen metabolism. Glutamate and glutamine are the primary products of assimilation and the main amino group donors. The available data suggests that the 2-oxoglutarate transport into chloroplasts occurs with glutamate export and involves two translocators [48]. It has been shown that the bulk of the label introduced as 2-[^{14}C]oxoglutarate was incorporated into glutamate and exported from chloroplasts [49]. It has been suggested that citrate, either stored in the vacuole or formed in the mitochondria, may be transferred to the cytoplasmic fractions for conversion to 2-oxoglutarate via cytosolic isoforms of AH and $NADP^+$–ICDH [50,45]. In relation to this it is interesting to note that Hanning and Heldt [51] suggested that the mitochondrial oxidation of malate in spinach leaves produces mainly citrate, which is converted to 2-oxoglutarate as the glutamate precursor. The expression analysis of a cytosolic $NADP^+$–ICDH from potato showed that the enzyme can be involved both in amino acid biosynthesis via the GS/GOGAT pathway, and in the cycling, redistribution and export of amino acids [52]. Evidently, the 2-oxoglutarate formation via $NADP^+$–ICDH and the ammonium assimilation via GS/GOGAT pathway is a possible regulated locus for coordination of carbon and nitrogen metabolism. It seems plausible that enzymes of this metabolic point may be co-regulated by way of molecular modifications or/and by changing of synthesis and degradation rates. In reality, it was found that Fd–GOGAT and $NADP^+$–ICDH might be co-regulated under the phytochrome control [53]. Some data indicate the existence of the coarse control of $NADP^+$–ICDH activity on the RNA level [52].

At the same time, the physiological role played by the chloroplastic and mitochondrial isoforms of $NADP^+$–ICDH remains totally unknown. It is suggested that, although a role in glutamate and glutamine synthesis for both isoforms cannot be totally excluded, there is no reason to believe that these forms provide the majority of the 2-oxoglutarate needed in chloroplasts [1]. Thus, cytosolic AH and $NADP^+$–ICDH might provide the chloroplastic and cytosolic compartments with the primary acceptor for reduced nitrogen and therefore be of crucial importance in the amino acid biosynthesis as well as in the NH_3 detoxification. In relation to this, glyoxylate condensation with succinate in ICL synthase reaction could be an additional source of isocitrate production needed for these processes (Fig. 2). However it can not be excluded that the transfer of citrate and isocitrate to cytosol from other cellular compartments, mediated by the special transport systems [36,37], may play a significant role for providing the necessary precursors of 2-oxoglutarate synthesis.

Citrate and isocitrate conversions in plant cells can be closely related to the fatty acid metabolism, when precursors of gluconeogenesis is produced from storage triacylglycerols through the fatty acid β-oxidation and the glyoxylate cycle. Thus, in plant tissues which contain the enzymes of the glyoxylate cycle as well as those of the TCA, the metabolism of citrate and isocitrate can follow two pathways. These metabolites may either be oxidised to CO_2 or converted to a four-carbon compound which can be metabolised further to sugar in seeds rich in fat. For example, in the endosperm of germinating castor beans, a simple competition between the ICDH and ICL would result in only a quarter of the iscocitrate being oxidised [54]. There are indications in recent papers that AH linked with the glyoxylate cycle in fatty seedling cotyledons is cytosolic rather than glyoxysomal [22,55,23,56]. Therefore, it is proposed that the citrate produced by the glyoxysomal CS must move to the cytosol to be transformed into isocitrate by the cytosolic AH. Then, the isocitrate should either return to the glyoxysomes to support the glyoxylate cycle activity, or be metabolised by the cytosolic $NADP^+$–ICDH [57]. The control of this branchpoint, linked with two possible fates for the isocitrate, might provide a means of regulating isocitrate and citrate utilisation. On the one hand, the use of isocitrate in glyoxylate cycle reactions will support the gluconeogenesis. On the other hand, the oxidation of isocitrate by ICDH might provide reducing equivalents and 2-oxoglutarate, which can be used as a carbon skeleton for amino acid biosynthesis or an intermediate of the TCA. Therefore, the control of this branchpoint through the regulation of ICDH and/or ICL activities can provide the cellular metabolism with flexibility in the utilisation of isocitrate (see below).

We may speculate that, the possibility of isocitrate distribution from the single metabolic point between

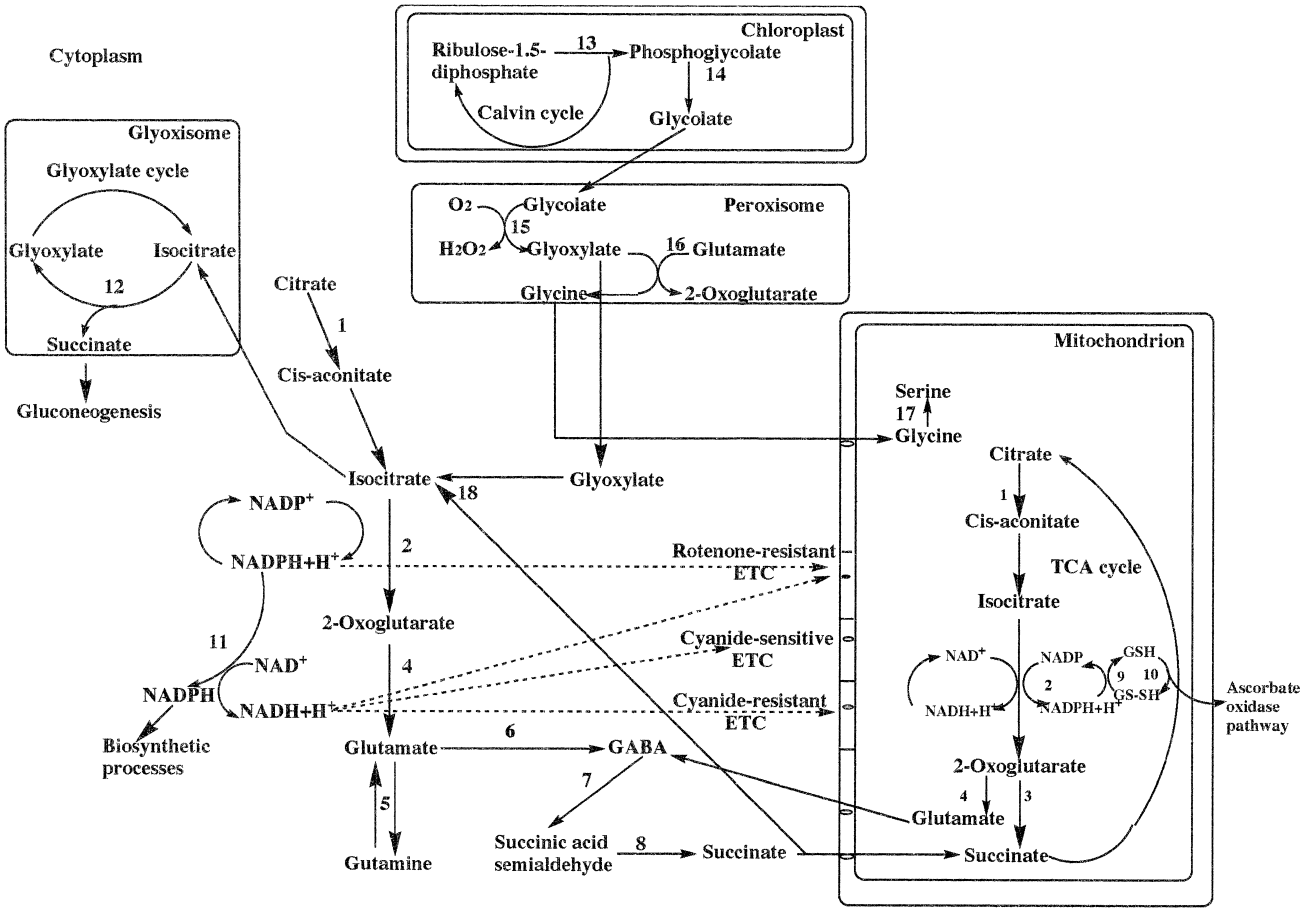

Fig. 2. Scheme of citrate and isocitrate conversions as related to the various metabolic processes in plants. Enzymes: (1) aconitate hydratase; (2) isocitrate dehydrogenase; (3) 2-oxoglutarate dehydrogenase complex; (4) glutamate dehydrogenase; (5) glutamine synthetase/glutamate synthase pathway; (6) glutamate decarboxylase; (7) GABA transaminase; (8) succinic semialdehyde dehydrogenase; (9) glutamate reductase; (10) ascorbate oxidase; (11) transhydrogenase; (12) isocitrate lyase (glyoxysomal); (13) ribulose-1,5-diphosphate carboxylase; (14) phosphoglycolate phosphatase; (15) glycolate oxidase; (16) glutamate–glyoxylate aminotransferase; (17) serine hydroxymethyl transferase; (18) isocitrate lyase (cytoplasmic). Dashed lines show interactions of oxidative processes with ETC.

different pathways is a reasonable requirement for an effective coordination at this step of cellular metabolism. Therefore, the AH absence in glyoxysomes is a particularly well suited circumstance for this. Although there are indications about the presence of AH activity in glyoxysomes, for example, in sunflower cotyledons, maize leaves ($\approx 7\%$ and $\approx 2\%$ of total activity, respectively) [[7], and references therein], it can not be excluded that is associated with possible cross-contamination of cellular fractions, which probably occurs in connection with the heterogenity of mitochondria and glyoxysomes. Further investigations must elucidate the problem of AH localization and its participation in processes of isocitrate distribution at this metabolic branchpoint.

Another very evident route of citrate conversions is the cleavage by ATP–CL. This enzyme is found in mango fruit, castor bean seeds, soya beans, maize and watermelon sprouts [58–60]. It has been suggested that the ATP–CL reaction may play an important role in fatty acid biosynthesis. Acetyl–CoA formed from citrate during catalysis by ATP–CL might be carboxylated by acetyl–CoA carboxylase to form malonyl–CoA, which, in turn, is used as the building blocks for fatty acid biosynthesis. An alternative source for acetyl–CoA production may be pyruvate. However, it was found that the activity of

PDH in plastids is considerably lower than in mitochondria, and approximately three times lower than ATP–CL activity [58,61]. Furthermore, the activity of PDH in the isolated chloroplasts of some species is insufficient to account for rates of fatty acid synthesis [[62], and references therein]. The available data allow us to conclude that ATP–CL could be of great significance for acetyl–CoA production especially under conditions in which respiratory carbon metabolism is restricted. However, it should be noted that many aspects of the functioning of ATP–CL in plants (e.g., its distribution in different tissues and intracellular localisation, activity regulation, physiological role, etc.) are still unclear.

It seems evident that a sufficient part of the production of secondary metabolites is associated with citrate and isocitrate metabolism. In relation to this an important role belongs to acetyl–CoA which is precursor of isoprene and isopentylpyrophosphate, these being key intermediates in the biosynthesis of terpens, alkaloids, certain phenolic compounds, etc. The role of secondary plant metabolites is very heterogeneous. But, interestingly, there are some indications that secondary pathways may still be physiologically important as a means of channelling and storing carbon compounds during periods when nitrogen is limited. It has also been suggested that large increases in the amount of secondary intermediates can occur in plants under conditions of stress [63].

Isocitrate conversions can be also connected with the metabolic pathway leading to the synthesis of γ-aminobutyrate. The accumulation of γ-aminobutyrate is associated with a variety of stress conditions including hypoxia, cold and mechanical damage [64–66]. A product of ICDH reaction, 2-oxoglutarate may be used by GDH for the production of glutamate, which can be directly decarboxylated via GDC to γ-aminobutyrate. The γ-aminobutyrate may be further converted to succinic semialdehyde and succinate by γ-aminobutyrate transaminase and succinic semialdehyde dehydrogenase, respectively. The conversions of glutamate and γ-aminobutyrate constitute a metabolic shunt which is interconnected with the TCA through succinate and 2-oxoglutarate. This feature determines a close metabolic relation between citrate and isocitrate conversions and the functioning of the γ-aminobutyrate shunt.

Thus, it is obvious that citrate and isocitrate conversions play a significant role in the formation of the general structure of the metabolic pathways in plant cells (Fig. 2). The citrate and isocitrate enzymatic transformations are the major generators of several main metabolic compounds, which participate in important biochemical pathways. Both the direction and the power of citrate and isocitrate flows may have a determining effect on cellular metabolism. The branchpoint of the most important metabolic pathways—the TCA and the anabolic and catabolic processes associated with the cycle, carbon and nitrogen metabolism, fatty acid oxidation and biosynthesis—exists at the stage of citrate and isocitrate conversions. Furthermore, some alternative pathways of compensatory significance can be linked with this metabolic stage as well. The coordination and distribution of biomolecular fluxes between the main metabolic pathways is apparently possible due to the changes in the intensity of citrate and isocitrate enzymatic conversions (see below).

4. Citrate and isocitrate conversions and the operation of the electron transport chain in plants

The plant ETC has some unique features. One of them is a cyanide-resistant nonphosphorylating electron transport pathway. The cyanide resistant system consists of a branchpoint from the conventional electron transport system, beginning with ubiquinone and terminating with alternative oxidase, distinct from cytochrome oxidase [67–70]. The alternative terminal oxidase is not blocked by CO, N^{3-}, or CN^{-}, but is inhibited by SHAM [71]. Another important feature of the plant respiratory chain is its ability to oxidise external NAD(P)H, and the operation of a rotenone-insensitive oxidation site [72,73]. The physiological importance of these mitochondrial features of plants is not yet clear. The oxidation of one NADH can yield up to 3 ATP if the electron transport is coupled entirely to cytochrome oxidase [72]. But, in the case of a bypass of complex I via a rotenone insensitive site [74] and a bypass of complexes III and IV via the cyanide-resistant oxidase [72], the yield of ATP can vary between 0 and 3 per NADH oxidised. Under certain physiological conditions (for example, under sufficient supplies of ATP and reducing equivalents by photosynthesis), the operation of the TCA pro-

vides intermediates for assimilatory pathways [75]. Of great importance in this regard are the metabolic conversions of tricarboxylic acids, occurring in the cytosol [[45,3], and references therein] and the oxidative processes unrelated to ATP synthesis, such as the oxidation of respiratory substrates by NAD(P)H-dehydrogense on the outer surface of the mitochondrial internal membrane [76,72,77], by cyanide-resistant oxidase [76,78] and by some other electron donor–acceptor systems (for example, the ascorbate oxidase system) [79,84]. Apparently, the operation of alternative mechanisms for NAD(P)H oxidation is essential, because the accumulation of reducing equivalents inhibits dehydrogenases, thereby hindering important biosynthetic processes [75].

The routes for citrate and isocitrate conversions are linked with the major metabolic pathways and, apparently, are under a fine control according to the specific demands and physiological status of the plant cell. As far as this is connected the coordinated operation of the electron transport systems in the cell is especially important, because it allows the involvement of citrate and isocitrate not only in catabolism, but also in assimilatory metabolism. It was shown by Rasmusson and Moller [80] that the respiration of malate- and isocitrate-oxidizing potato mitochondria can be enhanced by adding $NADP^+$ under conditions where external NADPH oxidation is suppressed. Therefore, it can be concluded that electrons cycled through NADPH are entering the mitochondrial ETC, either directly through complex I, or via the nicotinamide nucleotide *trans*-hydrogenase, producing NADH. In relation to this it was suggested by McIntosh and Oliver [81], that the feed-back inhibition of ICDH activity by NADH and NADPH seems to favour the effective control and balance the production of NADH and NADPH.

It has been shown that the suppression of electron flow at the various sites of ETC affected utilisation of 6-[^{14}C]-citrate in the leaves of maize and wheat [82]. Inhibition by KCN and SHAM suggests that the metabolic conversion of 6-[^{14}C]citrate in these plants is related to the functioning of both the cyanide-sensitive and the cyanide-resistant electron transport systems. It seems possible that the inhibition of complex I in the mitochondrial respiratory chain is accompanied by the activation of electron transport through the rotenone-resistant NADP(P)H-oxidising system. This view is consistent with the data on rotenone-resistant electron transport obtained by Moore et al. [83]. The oxidation of cytoplasmic NAD(P)H when complex I in the electron transport chain is inhibited can be performed by rotenone-resistant NAD(P)H-dehydrogenases. This route may be essential for involving citrate and isocitrate in the biosynthetic processes. As is well known, citrate conversions under these conditions can be mediated by cytoplasmic isoforms of AH and $NADP^+$–ICDH [50,24,84,45,3]. The effective operation of $NADP^+$–ICDH requires the rapid reoxidation of the newly formed reducing equivalents, because the optimum reaction rate is achieved at a certain level of oxidised NADP and because the inhibitory effect of NADPH on plant $NADP^+$–ICDH takes place [[1,3], and references therein].

The available data suggest that the level of citrate and isocitrate is significant for the regulation of mitochondrial AOX in plants. It is known that high AOX activity is dependent on the reduction of the covalently associated form to the noncovalently associated dimer, and the latter form can be activated by pyruvate [85,158]. Experiments with leaf mitochondria of transgenic *Nicotiana tabacum* show that AOX reduction can be mediated by intramitochondrial reducing power generated by the oxidation of TCA intermediates, especially, citrate, isocitratre and malate [138]. This mechanism may have an important role in the regulation of the partitioning of electrons to AOX. It was found that reduced protein has a higher affinity for ubiquinone [85]. Thus, we can speculate that the level of isocitrate and citrate oxidation in plant mitochondria may be a regulatory signal for the operation of the cyanide resistant electron transport system. Eventually, this might promote effective coordination of catabolic and anabolic processes in plant cells.

It is known that oxidative decarboxylation, which transforms isocitrate to 2-oxoglutarate, can be coupled with enzymatic conversions of oxidised and reduced forms of glutathione. In this oxidative system, the ascorbate oxidase functions as a terminal oxidase [88,89,82]. The oxidation of NAD(P)H, the product of the ICDH reaction, by the cytosolic ascorbate oxidase system can represent an effective route for oxidising the excessive amount of reducing equivalents produced in the course of citrate- and isocitrate-mediated processes.

It has been proposed that the inhibition of cyanide-sensitive and cyanide-resistant electron transport accelerates the utilisation of citrate in reactions of the γ-aminobutyric acid pathway which shunts the TCA. Isotopic studies of metabolic conversions of 5-[^{14}C]glutamate in leaves of wheat and maize have demonstrated that the electron transport inhibitors stimulated formation of γ-aminobutyrate, succinate and fumarate; and this effect was caused by the accelerated operation of the γ-aminobutyric shunt [90]. Apparently, under the conditions of an inhibited electron transport chain, the compensatory pathways exhibiting a certain autonomy may produce the pool of readily mobilised substrates for the TCA.

It can be concluded that the coupling of citrate and isocitrate metabolism with essential cellular processes and various electron transport pathways allows the fine control of metabolic conversions of citrate and isocitrate, according to the requirements of the plant (Fig. 2). The optional oxidation of citrate and isocitrate through several electron transport systems—cyanide sensitive, cyanide and rotenone resistant, and ascorbate oxidase systems—ensures the effective balancing of cellular $NAD(P)^+/NAD(P)H$ and ATP/ADP ratios, and consequently, coordinates the metabolic processes involving citrate and isocitrate, in accordance with specific conditions.

5. Citrate and isocitrate regulation of plant metabolism

Citrate and isocitrate and metabolites related to their conversions are known as potent effectors of many enzymes, associated with different metabolic pathways.

It may be suggested that citrate and isocitrate can influence the glycolysis process. In plants, substantial evidence indicates that PK and PFK are the primary control sites of the glycolytic flux to pyruvate [91–96]. As is well known, glycolysis can be inhibited by a high concentration of the TCA metabolites. Citrate can play a regulatory role in relation to both PFK and PK, since it acts as a negative effector of the enzymes [99,100].

Citrate and isocitrate may also be related to respiratory reactions by the affection of the enzymes of the TCA. The entry point of carbon into the cycle is the mitochondrial PDC, which catalyses the conversion of pyruvate to acetyl–CoA. It is known that this complex is regulated by reversible phosphorylations/dephosphorylations [101]. Steady-state PDC activity depends on the equilibrium between PDH kinase and phosphopyruvate dehydrogenase [102]. PDH kinase phosphorylates and thus activates the complex. Citrate as well as acetyl–CoA and NADH inhibit the PDH kinase [101].

It can be proposed that products of the mitochondrial PDC are required for metabolic reactions outside the mitochondria [[103], and references therein]. On the one hand, citrate can be used as a substrate of cytosol AH and, then may be further converted to 2-oxoglutarate by $NADP^+$–ICDH. On the other hand, citrate might metabolise by an ATP–CL, catalysing the cleavage of citrate to oxaloacetate and acetyl–CoA. It has been shown that acetyl–CoA can be converted to acetate by an A–CoAH, studied, to some extent, in fungi *Aspergillus niger* [104,105], in fruits of lemon [106], etiolated barley seedlings [107] and pea [108]. In fatty acid synthesis by chloroplasts, acetate has been found as one of the precursors [103]. It can, therefore, be concluded that citrate influences the activity of PDC, as well as fatty acid synthesis and 2-oxoglutarate formation in cytosol by AH and $NADP^+$–ICDH.

It should be noted that citrate, being a positive effector of NAD^+–ICDH, can play a significant role in the regulation of the TCA [[3], and references therein]. NAD^+–ICDH is often considered as the rate-limiting step in the TCA intensity in plant mitochondria [109,110]. It is interesting to note that the available data suggest significant differences between the regulatory proprieties of plant NAD^+–ICDH and those of enzymes from other sources. Thus, ADP and AMP are allosteric activators of the enzymes from some microorganisms and animal tissues [[1,3]; and references therein]. But, evidently, adenosine mono- and diphosphates are not directly involved in the regulation of plant NAD^+–ICDH. A significant role in the regulation of the catalytic activity of the enzyme is played by the NAD^+/NADH ratio, NADPH, citrate and isocitrate [81,97,98]. Studies of the binding of NAD^+–ICDH with isocitrate showed that the enzyme has four binding sites [111,97,98]. Two contact sites participate in catalysis and the other two act as effectors. It should be noted that substrate inhibi-

tion occurs at high isocitrate concentrations [97,98]. Citrate activates the enzyme from a number of sources [111,97,98]. However, the effect of citrate is significantly influenced by the pH of the medium and the isocitrate concentration. It is supposed that citrate may be bound to the same ligand/binding centre as isocitrate [111]. This might explain the inhibitory effect of citrate at high concentrations over the entire pH range [97]. It is possible that citrate and isocitrate can take part in dissociation/association effects occurring between the enzyme subunits (see below). Evidently, significant modifications of the NAD^+–ICDH functional state may occur due to cooperative binding of isocitrate, substrate inhibition, and the pH-dependent activation of an enzyme by citrate, establishing the important regulatory control of oxidative metabolism and energy generation in plant cells.

The $NADP^+$–ICDH activity in the course of both oxidative decarboxylation of isocitrate and the reductive carboxylation of 2-oxoglutarate can also be regulated under the influence of citrate. In this case, citrate inhibits the activity of the enzyme [112,11,113,114]. It is interesting that citrate has an opposite effect on plant NAD^+– and $NADP^+$–ICDH. It seems likely that the regulatory pattern of NAD^+– and $NADP^+$–ICDH's activities through citrate supports their function to some extent in providing the regulation of the intensity of both biosynthetic and energy metabolism.

Citrate can take part in the regulation of isomerisation reactions between *cis*- and *trans*-aconitate catalysed by aconitate isomerase [6]. *cis*- and *trans*-aconitate, in turn, may influence the activity of certain enzymes. Although the role of *cis*- and *trans*-aconitate in the regulation of enzymatic processes has virtually remained unexplored to this day, those compounds are known to influence the activity of ICL [115], AH [7] and NAD^+– and $NADP^+$–ICDH [98,113,114].

Fluorocitrate is known to be a powerful competitive inhibitor of AH [50,24]. Hydroxycitrate acts as a specific inhibitor of plant ATP–CL [58].

Thus, the intermediates linked with citrate and isocitrate conversions can play an important role in the regulation of cellular metabolism. On the one hand, these metabolites are substrates of key enzymes of the central metabolic pathways. On the other hand, they act as potent effectors of some regulatory stages in plant cell metabolism. The concentration of these intermediates, their distribution and transport between the different cell compartments apparently can affect the intensity of the main metabolic flows.

6. The role of citrate and isocitrate conversing enzymes in plant metabolism regulation

Enzymes linked with citrate and isocitrate conversions are directly involved in the regulation of ATP synthesis as the important points of control of the TCA. CS catalyses a crucial stage within the TCA-cycle, and it is the only enzyme in the cycle that can catalyse the formation of a carbon–carbon bond. It has been shown that CS from plants consists of two identical subunits with a combined molecular weight of about 100 kD [116]. CS is highly specific to its substrates. The enzymatic reaction has been demonstrated to proceed in accordance with an ordered mechanism. Oxaloacetate binds first with the enzyme, increasing the binding constant for acetyl–CoA. The available data suggest that the ATP/ADP ratio plays a key role in the regulation of mitochondrial CS activity. As the ATP concentration becomes higher, it causes the inhibition of CS [19,20]. Furthermore, CS activity is regulated by the changes in redox potential and the concentrations of citrate and 2-oxoglutarate [117,118,86].

As has been considered above, the other limiting step of the TCA is the NAD^+–ICDH reaction. Unfortunately, molecular and kinetic characteristics of plant NAD^+–ICDH have not been researched as much as the enzymes from animal tissues and some microorganisms. The reason for this is the extreme instability of the plant enzyme and its tendency to form aggregates with high molecular weight (see below). It has been reported that NAD^+–ICDH is an octamer of 41 kD monomers in beef heart [119]. The enzyme from this source was also observed in higher molecular weight forms apparently composed of two and four octamers. The molecular weight of NAD^+–ICDH from baker's yeast is 300 kD. The enzyme is composed of eight subunits with a molecular weight 39 kD [120]. However, yeast NAD^+–ICDH can also exist in different oligomeric forms with various molecular weights [121]. The subunit structure of

plant NAD^+–ICDH has not been determined exactly. However, in keeping with the available data which indicates that the oligomeric level of the enzyme changes during storage, purification and some manipulation, it can be concluded that the molecular characteristics of plant NAD^+–ICDH are very similar to those of enzymes from yeast and animal tissues. It was shown that pea NAD^+–ICDH can be eluted from Superose with apparent molecular weights of 1400, 690 and 300 kD [81]. It has been proposed that the molecular weight of the monomer for the enzyme is 47 kD. The molecular mass of the NAD^+–ICDH from pumpkin cotyledons as determined by gel filtration is 340 kD [97]. However, Cox and Davies [111] reported the molecular weight of pea NAD^+–ICDH, to be somewhat lower, 260 kD. This value was obtained in the presence of an activator, citrate. In the absence of citrate, the molecular mass increased to 535 kD, evidently due to aggregation. It was found that the degree of allosterism for NAD^+–ICDH is usually related to oligomer status [81]. Thus, it can be concluded that plant NAD^+–ICDH has oligomeric structure, indicating the possibility of dissociation/association processes occurring between its specific subunits. The degree of dissociation/association may vary depending on different ligands and environmental conditions, resulting in a change in the catalytic properties of the enzyme. Therefore, NAD^+–ICDH association/dissociation effects may have a regulatory function. The available data suggest that principal control over the activity of plant NAD^+–ICDH should be exercised through changes in the NAD^+/NADH ratio and citrate and isocitrate concentrations. In addition, fluctuations in the level of several organic acids, most notably 2-oxoglutarate, glyoxylate, oxaloacetate, *cis*- and *trans*-aconitate might also be implicated in altering enzyme activity [111,81,97,98]. The specific characteristics of the molecular structure and modifications of NAD^+–ICDH activity affected by cellular intermediates probably provide for the fine regulation of the TCA operation, depending on certain metabolite concentrations.

There are some indications that show the association of TCA enzymes form clusters and metabolons which ensure structural–functional unity for the corresponding enzyme systems [122,123]. It has been demonstrated that a triple complex of NAD^+–ICDH, 2-oxoglutarate dehydrogenase and NADH–ubiquinone oxidoreductase can be formed [124]. This is probably very important for the effective transport of intracellular intermediates between different steps of the metabolic pathway. However, there is a need for more studies of the interactions between TCA enzymes, as well as of the mechanisms of their regulation by reversible adsorption on subcellular structures in plant mitochondria.

A dimer structure was established for cytosol $NADP^+$–ICDH from different plant sources [125,26,27,34,113]. The enzyme consists of two identical subunits with a molecular weight of about 42–45 kD. The $NADP^+$–ICDH activity can be regulated under the influence of a number of organic acids (2-oxoglutarate, citrate, *cis*- and *trans*-aconitate, glyoxylate and oxaloacetate), ions of metals (Mn^{2+}, Mg^{2+}, Zn^{2+}, etc.), and some nucleoside phosphates [[1,3], and references therein]. It is suggested that the activity of $NADP^+$–ICDH from *Escherichia coli* is controlled by the phosphorylation/dephosphorylation mechanism [126–130]. However, in plant organisms, particularly in both sorghum leaves and soya bean nodules, no covalent modification of $NADP^+$–ICDH by phosphorylation was observed [[1,3], and references therein].

The existence of a set of various regulatory mechanisms affecting the activity of NAD^+– and $NADP^+$–ICDH is apparently connected with the coordination of the intensity of biosynthetic and catabolic processes in a cell. It has been suggested that the regulation of the activity of NAD^+–ICDH from *Acetobacter peroxydans* by AMP is associated with the distribution of cellular citrate resources between NAD^+–ICDH and $NADP^+$–ICDH [131]. The importance of this hypothesis lies in the fact that a decrease in ATP concentration and the corresponding increase in AMP level in the cell will favour the NAD^+-specific enzyme. Therefore, the number of electrons being transferred from isocitrate to the respiratory chain increases along with the synthesis of ATP during oxidative phosphorylation. However, the plant NAD^+–ICDH appeared insensitive to the action of AMP and ADP [9,81,97,98]. We may speculate that under specific conditions of $NADP^+$–ICDH retardation, a decrease in the isocitrate conversion rate may shift the AH equilibrium toward citrate formation. It has been demonstrated that citrate can activate

plant NAD^+–ICDH [22,156,97,98]. The AH activity and the ratio of citrate and isocitrate levels are known to be of importance for the regulation of NAD^+–ICDH activity also [111,156,97]. It should be noted that citrate can be actively accumulated by plant mitochondria [36,132]. Thus, retardation of the $NADP^+$–ICDH reaction sets the stage for an increase in the rate of the utilisation of respiratory substrates by NAD^+–ICDH (Fig. 3). Therefore, a shift of cellular metabolism toward catabolic processes becomes possible. So regulatory mechanisms of NAD^+ and $NADP^+$–ICDH activities can promote a fine coordination of catabolism and anabolism, because two types of ICDHs can participate in these processes concurrently.

A number of papers point out that cytosolic and chloroplastic $NADP^+$–ICDH are distinct isoenzymes [25,133]. Chloroplastic $NADP^+$–ICDH seems to be a dimeric protein with a molecular weight of 136 kD. On the one hand, it has been suggested that the reaction promoted by chloroplastic $NADP^+$–ICDH produces 2-oxoglutarate necessary for the biosynthesis of glutamate in chloroplasts [28]. On the other hand, because of the evidence that a very low activity of chloroplastic $NADP^+$–ICDH takes place in leaves ($\approx$ 4–5% of the total), it has been proposed that this activity is not capable of supplying the 2-oxoglutarate required for the GS/GOGAT pathway operation in chloroplasts [52]. The accumulated evidence favours the chloroplastic isoenzyme-catalysed reaction as being the site of production of at least part of the reducing power needed for biosynthetic processes in the chloroplast when photosynthetic NADPH generation is low [134,135].

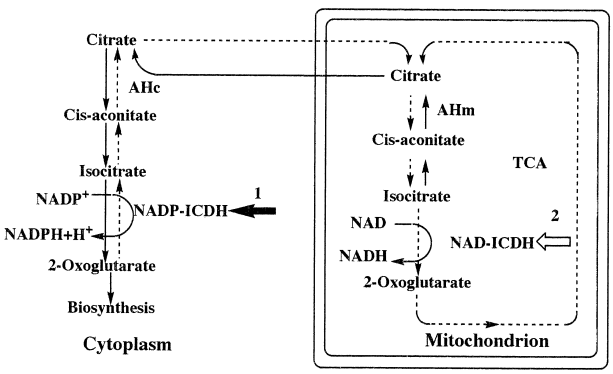

Fig. 3. Hypothetical scheme of coordination of energy and biosynthetic metabolism at the level of NAD^+– and $NADP^+$–ICDH in a plant cell. Dashed lines show the increase of TCA operation after retardation of $NADP^+$–ICDH reaction: 1—inhibition of $NADP^+$–ICDH under some conditions (higher concentrations of citrate, 2-oxoglutarate, etc.); 2—activation of NAD^+–ICDH by citrate.

The question of the physiological role of mitochondrial $NADP^+$–ICDH in plants remains open. The $NADP^+$–ICDH from pea mitochondria seems to be a protein of 94 kD. This isoenzyme has similar, as well as different, properties when compared with the cytosol isoenzyme [30]. Some alternative functions for the mitochondrial $NADP^+$–ICDH have been proposed. One hypothesis is that the enzyme activity could be coupled to a transhydrogenase. According to Smith and Plaut [136], in mitochondria from animal tissues $NADP^+$–ICDH can participate in regulating the transhydrogenase reaction, which catalyses the NADPH-dependent NAD^+ reduction. As it is known, the NAD^+/NADH ratio is an important regulatory factor for plant NAD^+–ICDH [81,97,98]. Thus, it seems plausible that $NADP^+$–ICDH may be involved in the regulation of the TCA in plants via the cascade mechanism described above, the most important stage of which is the reaction catalysed by transhydrogenase. However, the operation and regulation of transhydrogenase in plants have not been established. $NADP^+$–ICDH activity has also been proposed as controlling glycolysis by changing the citrate level [137]. However, taking into consideration the experimental evidence for a significant role of citrate and isocitrate oxidation for AOX function, we may conclude that mitochondrial $NADP^+$–ICDH might participate in the regulation AOX action. The most likely explanation of the influence of citrate, isocitrate and malate on the AOX function is that intramitochondrial reducing equivalents provided by the activity of ICDH or malate dehydrogenase are able to promote the AOX reduction [138]. Furthermore, extramitochondrial NADH and NADPH were ineffective in aiding the modification of AOX to a more active form. It should be noted that some articles indicate that NADPH is the required substrate for AOX reduction, because malate oxidation by malic enzyme, which provides NADH, had the lesser ability for the protein activation. Thus, the available data are pointed out a real possibility for mitochondrial $NADP^+$–ICDH to participate in determining the partitioning of electrons to the cyanide resistant path-

way. Another proposed function of the enzyme is the supply of NADPH for glutathione reduction in the matrix. Therefore, the isoenzyme might be implicated in the removal of activated oxygen species [45]. It seems plausible that participation of $NADP^+$–ICDH in the removal of activated oxygen species might be an important factor for the promotion of homeostasis of plant cells. In relation to this interestingly, that plant AH is rapidly inactivated by hydrogen peroxide [139]. However, to date, the experimental evidence for the physiological role of mitochondrial $NADP^+$–ICDH is not quite available, and thus, all hypotheses considered above are a speculative proposals.

Unfortunately, as yet little is known about plant AH. However, there is evidence that the intensity of the TCA, as well as some biosynthetic processes, also depend on the activity of AH, present in plants in multiple forms, with different regulatory and kinetic properties with respect to citrate, isocitrate, *trans*- and *cis*-aconitate [24,7]. It has been shown that AH is a monomeric enzyme. The molecular weight of AH from plant sources has been reported to be 90–100 kD [24,139]. It has been demonstrated that different AH have close molecular weights [24]. At the present time AH claims special attention because it apparently may serve as an iron-responsive regulator of mRNA translation or stability, as well as an enzyme [140,141]. Unfortunately, plant AH has not been studied with respect to this. Because the AH participating in the glyoxylate cycle seems to be localised in the cytosol [23,56] it has been suggested that the isocitrate formed by such AH could easily be used by a cytosolic $NADP^+$–ICDH for 2-oxoglutarate production. Therefore, the rates of the glyoxylate cycle and TCA are controlled at the isocitrate metabolism stage since representative enzymes for each pathway, ICL and ICDH, compete for the same substrate. In fact, the physiological importance of the phosphorylation/dephosphorylation of $NADP^+$–ICDH in *Escherichia coli* is in the distribution of isocitrate resources between the TCA and glyoxylate cycles during the cultivation of the bacterium on acetate [126]. On the basis of the available data it can be proposed that the mechanisms controlling plant $NADP^+$–ICDH activity appear to function primarily at the level of differential isoenzyme expression rather than through phosphorylation/dephosphorylation. The presence of tissue-specific isoenzymes in plants with an active glyoxylate cycle suggests regulation by gene expression [125,57]. Some data suggest the simultaneous involvement of $NADP^+$–ICDH and glyoxylate cycle enzymes in isocitrate conversions. For example, during the growing of *Chlamydomonas reinhardtii* cells in darkness, with acetate as the carbon and energy source, an enhancement of $NADP^+$–ICDH activity was observed, together with ICL induction [142]. This suggests the existence of a coordinate expression of the two enzymes. Some data indicate that the variation in specific activity could be accounted for by the coarse control of enzyme synthesis through transcription and/or translation events [128,143,144]. Effects of various intermediates on isocitrate metabolising enzymes suggest that a fine-control mechanism may also be operable in controlling carbon flux through different isocitrate-converted pathways. ICL has been purified and characterised from several plant sources, where it appears to be a tetrameric protein of 30 kD monomers which is sensitive to the influence of the intermediates of both TCA and glyoxylate cycles [[87,7,145], and references therein]. For example, citrate and *cis*-aconitate are inhibitors of ICL from sunflower cotyledons [145,115]. However, the inhibiting action of *trans*-aconitate is considerably weaker. The reaction products of ICL, glyoxylate and succinate, as well their analogs (for example, itaconate), can also inhibit enzymes. As considered above, ICDH activity is also subject to metabolic regulation, and intermediates of the glyoxylate cycle have been shown to alter its activity, at least in part [[1,3], and references therein]. It should be noted that glyoxylate and oxaloacetate, which are present in the reaction mixture, when combined, have a very strong inhibitory effect on the $NADP^+$–ICDH from a number of sources, including plants. The physiological role of the inhibition of $NADP^+$–ICDH by oxaloacetate and glyoxylate is not well understood, but it was initially thought to be important for the regulation of the isocitrate branchpoint between the glyoxylate and TCAs. However, the synergism of the inhibitory effect of a mixture of oxaloacetate and glyoxylate was demonstrated also for $NADP^+$–ICDH from tissues lacking the glyoxylate cycle. Therefore, the question of the 'concerted inhibition' role remains open. But, it was found that a mixture of glyoxylate and oxaloacetate does not affect the $NADP^+$–ICDH from peas in the course of

the reverse reaction, e.g., the reductive carboxylation of 2-oxoglutarate [11]. Thus, an opposite action of glyoxylate and oxaloactate mixture on direct and reverse reaction of plant $NADP^+$–ICDH takes place. Therefore, a controversial proposal; could be suggested that these intermediates may influence the equilibrium of the enzymatic reaction and this mechanism of $NADP^+$–ICDH activity regulation might be one of a factors, which determine 2-oxoglutarate level in plant cells.

It is possible there are some other mechanisms controlling the enzymes associated with the isocitrate branchpoint. Thus, for example, during the germination of castor bean seeds, $NADP^+$–ICDH will have become unstable by the fourth day of germination under the effect of ricinoleate, then it can easily be inactivated by trypsin. However, $NADP^+$–ICDH extracted from a two-day old plant is both stable and trypsin insensitive [146]. Moreover, the content of ricinoleate begins to increase after two days of germination, which correlates with the decreasing stability of ICDH. Besides this, it is known that a degradation of ICL may be caused by endogenous inhibitors, as is characteristic for sunflower [157], flax [147] and hemp [7]. Taken together, the rates of glyoxylate and TCAs or amino acid production can be regulated by a distribution of isocitrate pools between ICL and ICDH.

The presence of ICL in plant tissues lacking the glyoxylate cycle has been definitely detected [148,12,149,13]. It has been proposed that the synthase reaction of this enzyme provides the source for reloading the pool of TCA intermediates (i.e., isocitrate) and is especially important for the function of the TCA during illumination, when glycolysis is suppressed [14,15]. This reaction may be a possible additional source of CO_2, arising out of photorespiratory processes [15]. Evidently, the synthesis of isocitrate from glyoxylate and succinate can provide the relation between the TCA and photorespiration [150,14] (Fig. 2).

That ATP–CL apparently contributes to the regulation of the fatty acid synthesis in plants cannot be excluded [58–60]. In this case, the inhibition of the enzyme activity by hydroxycitrate may have a regulatory function. However, because data concerning this enzyme are extremely rare, its regulation and physiological relevance is highly questionable.

In certain living organisms such as yeast, for example, the distribution of citrate pools between the TCA and fatty acid synthesis has been shown to depend significantly on the regulatory activity of the CS, ICDH and A–CoAC [151]. The effect of the adenylate charge energy of a cell is a major factor in this context. Increasing the ATP/ADP ratio decreases the affinity of the NAD^+–ICDH with respect to isocitrate, and it is accompanied by a shift in the AH equilibrium toward citrate accumulation. Citrate serves as a positive allosteric effector of the A–CoAC. As a result, the rate of the carboxylation of acetyl–CoA into malonyl–CoA increases, and, therefore, the enhancement of fatty acid synthesis becomes possible. At the same time, an increase in ATP concentration can provide a rise in CS K_m with respect to acetyl–CoA. Thereby, the flux of acetyl–CoA through the TCA decreases. The opposite takes place when the ATP/ADP ratio decreases.

At present, it cannot be concluded that a similar strategy of metabolic regulation might be possible in plants. Although A–CoAC may determine the intensity of fatty acid synthesis in plants, understanding the regulation of fatty acid metabolism requires a study of the factors controlling A–CoAC. As it is known, in animal tissues and fungi, A–CoAC is controlled by several mechanisms, including activation by citrate, feedback inhibition by acyl–CoA, and phosphorylation. However, none of these mechanisms has yet been demonstrated for plant enzymes [[62], and references therein]. But, it should be noted that the intensity of fatty acid synthesis in leaves, as well as A–CoAC activity, is higher in the light than in the dark. In contrast, according to some indications, the rate of the TCA in plants is lower in the light [39,51]. Furthermore, plant CS is considered a regulatory enzyme, subject to feedback inhibition by its own product and to control by the ATP level [117,19,20,118,86]. ATP can inhibit $NADP^+$–ICDH [125,113,114] as well as the NAD^+–ICDH from certain plants [97,98]. As for the activating effect of citrate on plant NAD^+–ICDH, it has been shown that this metabolite affected the enzyme in a very complex manner, depending on its own and the isocitrate's concentrations, as well as pH. For example, at the optimal citrate concentration (0.5 mM) the activity of pumpkin cotyledon NAD^+–ICDH is increased by $\approx$ 15% at pH 7.2 and by $\approx$ 70% at pH 8.0 [97]. As

was found, ATP–CL is most active in the pH range of 7.0 to 7.5 [58]. Hence, taking to consideration peculiarities of enzymes functioning in plant cells, we can speculate that a regulation of citrate distribution between the TCA and fatty acids synthesis might be possible, similarly as in yeast, because the regulatory proprieties of enzymes correspond in general to coordination scheme of the metabolic processes. However, more detailed studies are needed to elucidate the relevance of citrate and isocitrate producing and utilising enzymes in a complex system of coordination of fatty acid metabolism and the TCA or amino acid production.

Thus, it can be concluded that various forms of citrate and isocitrate conversing enzymes may take part in the cellular processes associated with the regulation of the energy and redox potentials and the main biosynthetic pathways. This may be achieved through modifications of key enzyme activities and their kinetic properties, depending on numerous control mechanisms.

7. Concluding remarks

A lot of research has focused on the organization and enzyme regulation of citrate and isocitrate metabolism in plant cells, but a number of aspects of this problem remain unknown. It has, nevertheless, become clear that citrate and isocitrate enzymatic conversions can play a major role in the distribution of biomolecular flows via the central metabolic pathways and certain associated secondary metabolism reactions. As an important metabolic branchpoint, citrate and isocitrate conversions may provide carbon skeletons for nitrogen assimilation and reducing equivalents for biosynthetic reactions, support the functioning of the glyoxylate cycle and the process of gluconeogenesis, and play an important role in the TCA and in energy metabolism as a whole (Fig. 4). Furthermore, citrate and isocitrate metabolic pathways may be a source of acetyl–CoA for the synthesis of fatty acids. The coupling of citrate and isocitrate metabolism with various electron transport systems (e.g., cyanide sensitive, cyanide and rotenone resistant, and ascorbate oxidase systems) ensures the effective balancing of the reducing power and the energy charge of the cell. The occurrence of diverse, rather effective mechanisms of activity regulation is characteristic of the citrate and isocitrate conversing enzymes. This suggests that the reactions of citrate and isocitrate transformations are an important stage, contributing significantly to the regulation of the direction of cellular metabolism. The coordination of energy and biosynthetic processes evidently may occur at the level of citrate and isocitrate distribution. However, the current hypothesis on the regulatory mechanisms requires further experimental confirmation. More studies concerning the principles of regulation based on the interaction of different enzymatic

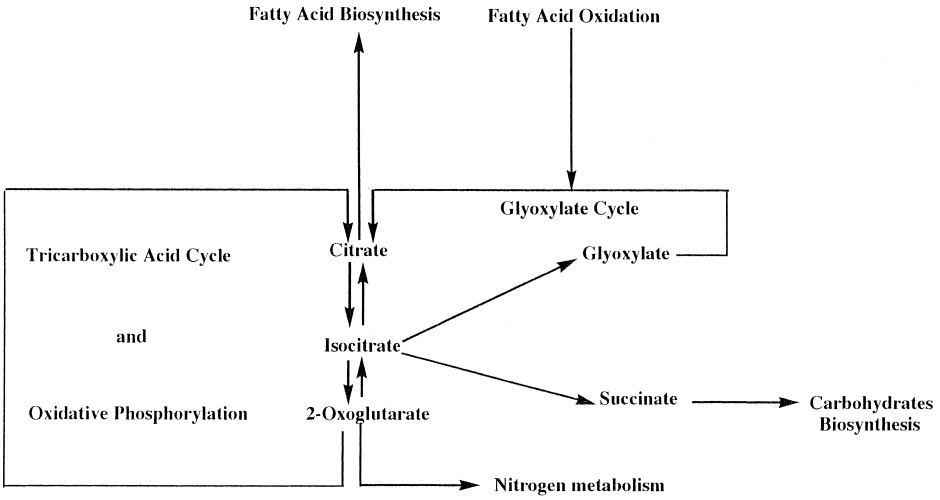

Fig. 4. Incorporation of the routes for citrate and isocitrate conversions into the system of cross-linked metabolic processes.

forms of citrate and isocitrate conversing enzymes are also needed. Unfortunately, owing to the low activity and stability of isoenzymes (e.g., cytosolic ICL, chloroplastic and mitochondrial $NADP^+$–ICDH, etc.), it is difficult to investigate such forms of the enzymes, using classical techniques. However, studies involving molecular biology approaches can elucidate the physiological role of the isoenzymes. The isolation and analyses of full-length cDNAs which encode isoenzymes, as well as the study of the regulation of activities and expression of the respective genes can open new possibilities for a deeper understanding of the physiological relevance of citrate- and isocitrate-conversing enzymes during plant development. Some data suggest the possibility that changes in metabolite levels in plant cells serve as a signal that regulates the genes which encode the enzymes of citrate and isocitrate transformations [52,152–155]. For example, it has been shown that nitrate or sucrose can induce a genetic expression of cytosolic $NADP^+$–ICDH in potatoes [52]. However, the major question remains unanswered. Is the control of enzymatic citrate and isocitrate conversions exerted primarily through the level of gene expression or the metabolic fine regulation of enzyme activity? The relative contributions of different mechanisms must be evaluated. Now, with the ability to manipulate expression in transgenic plants, new possibilities for the solution to this problem appear. Future research into the nature of the factors regulating the expression of enzymes under environmental conditions may provide important information for understanding their functions.

It should be noted that the intracellular compartmentalisation of enzymes and metabolites apparently plays a significant role in the coordination of citrate and isocitrate metabolism, as well. The transfer of citrate and isocitrate or their derivatives between different compartments and the operation of the respective membrane transport systems may be another mechanism controlling individual reactions as well as regulating interrelated metabolic pathways. Therefore, citrate and isocitrate metabolism play an important role in the development of interconnections between different cellular compartments.

It can thus be concluded that citrate and isocitrate conversions are incorporated into the system of cross-linked metabolic processes and are regulated by a number of factors. The general organisation of citrate and isocitrate metabolism, and the function and regulation of the related enzymes all point to a high coordination of the main processes in plant cells.

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