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An update on the role of mitochondrial α-ketoglutarate dehydrogenase in oxidative stress

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Abstract

The activity of mitochondrial alpha-ketoglutarate dehydrogenase complex (KGDHC) is severely reduced in human pathologies where oxidative stress is traditionally thought to play an important role, such as familial and sporadic forms of Alzheimer's disease and other age-related neurodegenerative diseases. This minireview is focused on substantial data that were accumulated over the last 2 decades to support the concept that KGDHC can be a primary mitochondrial target of oxidative stress and at the same time a key contributor to it by producing reactive oxygen species. This article is part of a Special Issue entitled ‘Mitochondrial function’.

Keywords

Mitochondria; Oxidative stress; Reactive oxygen species

Introduction

The mitochondrial α-ketoglutarate dehydrogenase complex (KGDHC) catalyzes the reaction

$$\alpha - \text{ketoglutarate} + \text{CoASH} + \text{NAD}^{+} \rightarrow \text{succinyl} - \text{CoA} + \text{CO2} + \text{NADH}.$$

It is located in the matrix of mitochondria where it is tightly associated with the matrix side of the inner membrane (Maas and Bisswanger, 1990). It binds specifically to Complex I of the mitochondrial respiratory chain (Sumegi and Srere, 1984) and may form a part of the tricarboxylic acid cycle (TCA) enzyme supercomplex (Lyubarev and Kurganov, 1989). The KGDHC is composed of multiple copies of three enzymes: α-ketoglutarate dehydrogenase (E1k component, EC 1.2.4.2), dihydrolipoamide succinyltransferase (E2k component, EC 2.3.1.12), and dihydrolipoamide dehydrogenase (E3 component, EC 1.6.4.3). The electrons from E1k reduce the lipoyl groups in E2k. E3 reoxidizes the reduced lipoyl groups ofE2k and transfers the electrons to NAD^+, forming NADH. The E1k and E3 components are non-covalently bound to a core formed by E2k components (Sheu and Blass, 1999; Wagenknecht et al., 1983). KGDHC is activated by low concentrations of Ca^{2+} (10^{-7}–10^{-5} M) and ADP (~10^{-4} M for half-maximum activation) (Lawlis and Roche, 1981; McCormack and Denton, 1979), and it is inhibited by high NADH and its own product, succinyl-CoA (Hamada et al., 1975; Kiselevsky et al., 1990; LaNoue et al., 1983; McMinn and Ottaway, 1977; Wan et al., 1989). The latter is the key substrate for heme biosynthesis and also a substrate for the succinyl-CoA ligase, the only enzyme in brain mitochondria with the substantial capacity for ATP-generating substrate-level phosphorylation. Thus, KGDHC is important for generating ATP in the mitochondria in the absence of oxidative phosphorylation (Chinopoulos et al., 2010). The importance of KGDHC in TCA and overall energy and heme metabolism has had been extensively and repeatedly reviewed elsewhere (e.g., see Gibson et al., 2010); this

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minireview is focused primarily on the properties of KGDHC as the target of oxidative stress and its E3 component as a source of reactive oxygen species.

Why is studying KGDHC important?

The interest in KGDHC activity in relation to human pathology and specifically brain metabolism stems from the fact that its activity is severely reduced in human pathologies where oxidative stressis traditionally thought to play an important role. The KGDHC activity is reduced by ~57% in brains of patients with Alzheimer's disease as originally reported by Gibson et al. (1988) and later confirmed by several laboratories (Butterworth and Besnard, 1990; Mastrogiacoma et al., 1996; Mastrogiacomo et al., 1993; Terwel et al., 1998). Decreased KGDHC activities occur in genetic (Gibson et al., 1998) and sporadic (Albers et al., 2000) forms of AD. In early AD-type dementia, the neocortical metabolic abnormalities precede the non-memory cognitive defects (Haxby et al., 1986), thereby suggesting a role for dysregulation of metabolism in etiology of AD. KGDHC activity is also reduced in several other age-related neurodegenerative diseases including Parkinson's (Gibson et al., 2003), Huntington (Klivenyi et al., 2004), Wernicke Korskoff (Butterworth et al., 1993) and progressive supranuclear palsy (Albers et al., 2000; Park et al., 2001).

KGDHC is an easy target of oxidative stress

One of the reasons for this consistent deficiency of KGDHC in neurodegenerative diseases may be that KGDHC appears to be more sensitive to oxidative damage than most other mitochondrial proteins (Tretter and Adam-Vizi, 2000), perhaps due to the large number of sulfhydryl groups and tyrosines that are subject to oxidant modification whereas being critical for KGDHC enzymatic activity. Analysis of the human sequence shows that KGDHC has 37 cysteine residues (E1k has 21, E2k has 6 and E3 has 10); there are also multiple tyrosine residues in KGDHC (31 in E1k, 5 in E2k and 11 in E3). KGDHC is sensitive to oxidants that increase 4-hydroxy-2-nonenal (Humphries and Szweda, 1998) and elevate the protein carbonyl content (Cabiscol et al., 2000). A variety of oxidants inactivate KGDHC, such as H_2O_2 (Chinopoulos et al., 1999; Gibson et al., 2000; Nulton-Persson et al., 2003; Xu et al., 2001), peroxynitrite (Park et al., 1999), nitric oxide (Park et al., 1999), hydroxynonenal (Humphries et al., 1998), chloroamine (Xu et al., 2001), sodium hypochlorite (Xu et al., 2001), t-butyl hydroperoxide (Rokutan et al., 1987) and acrolein (Pocernich and Butterfield, 2003). Catecholamines enhance E3 inactivation by the copper Fenton system (Correa and Stoppani, 1996). Knockout of SOD2 in mice increases ROS production and inactivates E1k of KGDHC (Hinerfeld et al., 2004). Increases in cellular ROS and diminished KGDHC are seen following inhibition of respiration in microglia (Park et al., 1999) and increased MAO expression (Kumar et al., 2003). KGDHC also interacts with thioredoxin to maintain the mitochondrial sulfur redox (Bunik, 2003; Gourlay et al., 2003). Thioredoxin couples the redox state of the lipoyl groups in E2k to glutathione and peroxiredoxin (Bunik, 2003). The increase in the ratio of dihydrolipoate/lipoate in the E2k component reciprocally diminishes E1k activity by changing its cooperative properties (Bunik et al., 1989, 1990), inactivation (Bunik et al., 1997), production of ROS (Bunik and Sievers, 2002) and reduction of disulfides (Bunik et al., 1995). The thiyl radical of the E2k-bound dihydrolipoate induces the inactivation of E1k by one electron oxidation of the 2-oxo acid dehydrogenase catalytic intermediate. The inactivation prevents the transformation of the 2-oxo acids in the absence of terminal substrate, NAD^+ (Bunik and Sievers, 2002). Thioredoxin protects KGDHC from the E1k inactivation. In turn, the activity of thioredoxin and other 'oxins (peroxiredoxins, glutaredoxins, which are important in maintaining other sulfhydryl group-dependent enzyme activity) in mitochondria depends on the supply of reducing equivalents from TCA enzymes tothioredoxin reductase 2 that regenerates the 'oxins (Andreyev et al., 2005). This supply depends on the flux through TCA, which is in some tissues (such as the brain (Sheu and Blass, 1999)) controlled by KGDHC activity.

Thus, ample evidence support the concept that KGDHC is an easy target of oxidative stress because it seems to be especially vulnerable to oxidative damage while being crucially important for keeping the mitochondrial antioxidant defense system operational.

KGDHC is a significant source of ROS

Or, more precisely, the E3 component of KGDHC is a substantial source of ROS in mitochondria. Of note, the E3 component of KGDHC is genetically and physically identical to that of several other E3-bearing enzymes (pyruvate dehydrogenase complex (PDHC), branched-chain α-keto acid dehydrogenase complex (BCKDC), and the glycine cleavage complex; the other protein components are unique to E3-bearing enzyme complexes). E3 is known as dihydrolipoamide dehydrogenase or lipoamide dehydrogenase (commonly used abbreviations are DLD, DLDH, or LADH). For enzymatic activity, E3 requires non-covalently bound FAD (Koike et al., 1974; Patel and Roche, 1990; Reed and Hackert, 1990). E3 is the most abundant flavoprotein in muscle and brain mitochondria (Kunz and Gellerich, 1993). It is not known whether E3 is present as a stand-alone, non-complex-bound enzyme in the mitochondrial matrix. When isolated, E3 exhibits multiple enzymatic activities such as e.g., diaphorase (Massey, 1960) that is capable of reducing quinones at the expense of NADH, or proteinase (reviewed elsewhere (Gibson et al., 2010)). Again, it is not known whether E3 can catalyze these reactions when it is integrated in the proper enzyme complexes.

E3 can generate superoxide and hydrogen peroxide. KGDHC is the major source of H_2O_2 in the mitochondria in resting state when the ratio of NAD^+/NADH is low (Starkov et al., 2004; Tretter and Adam-Vizi, 2004; Tretter and Adam-Vizi, 2005). ROS production has been shown with isolated purified KGDHC (Starkov et al., 2004; Tretter and Adam-Vizi, 2004) and PDHC (Starkov et al., 2004) and in isolated mouse brain mitochondria (Starkov et al., 2004). Mice genetically rendered deficient in E3 by 50% by suppressing Dld gene expression exhibited about 55% of enzymatic activity of KGDHC in brain mitochondria (the activity of PDHC was depleted by only ~20%, likely because of the differences in the assembly of PDHC and KGDHC complexes). Brain mitochondria isolated from these $Dld^{+/-}$; mice exhibited about 50% less ROS production with α-ketoglutarate as well as with succinate.On the other hand, mice rendered deficient in KGDHC activity by suppressing the expression of the E2k component ($DLST^{+/-}$ mice) also exhibited about 58% of KGDHC activity in brain mitochondria (Yang et al., 2009), yet the ROS production was normal (these data were not published). This indicates that the source of ROS in KGDHC was E3 with electrons coming from either α-ketoglutarate or NADH (Starkov et al., 2004). It would also explain why the ROS production was suppressed when mitochondria oxidized the succinate in State 4 (Starkov et al., 2004). It is believed that under such conditions ROS are generated at Complex I of the respiratory chain because of reverse electron transfer from the succinate. However, the mitochondrial pyridine nucleotide pool becomes over-reduced under such conditions, which prompts ROS production from other enzymes capable of it which are linked to pyridine nucleotide pool,, such as KGDHC. Thus, ROS production in State 4 in mitochondria oxidizing succinate is a sum of Complex I-generated ROS and ROS generated by other enzymes linked to the NAD pool in the mitochondrial matrix.

Although E3 is a part of several enzyme complexes including KGDHC and PDHC, there is greater impairment of KGDHC than of PDHC activity in E3-deficient mitochondria (Klivenyi et al., 2004; Starkov et al., 2004). The data strongly indicate that KGDHC is a primary site of ROS production in normally functioning mitochondria (Starkov et al., 2004).

This ROS production appears to be pathologically important since inhibitors of KGDHC protect against in vitro hypoxia (Huang et al., 2005) and glutamate toxicity (Zündorf et al., 2009) in parallel with blocking ROS production.

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Isolated E3 also produces ROS (Bunik and Sievers, 2002). The ROS production from KGDHC is stimulated by low availability of its natural electron acceptor, NAD^+ (Starkov et al., 2004; Tretter and Adam-Vizi, 2004). The dependence on NAD^+/NADH provides a direct link to metabolism.

Mutations in the human Dld gene encoding E3 result in the inherited deficiency of the E3 subcomponent of KGDHC with severe or even lethal consequences. The disease-causing mutations involve either the cofactor-binding sites (I12T, K37E, G194C, M326V, I358T), the disulfide redox center (P453L) or the dimerization region (E340K, D444V, R460G) (see Ambrus et al., 2011 for the references). Recently, researchers led by Adam-Vizi (Ambrus et al., 2011) investigated the most important pathogenic mutants of Dld by using site-directed mutagenesis of human Dld; the mutated proteins were expressed in *Escherichia coli*, isolated, purified and studied with the help of an array of biochemical, structural and biophysical methods. The authors found that G194C, P453L, E340K and D444V mutants exhibited significantly enhanced ROS generation. It is interesting that the location of the mutation in E3 or the conformation changes in the enzyme caused by these mutations had no apparent relation to the enhanced ROS generation. Two ROS-enhancing mutations were at the homodimer interface (E340K, D444V), one at the disulfide-exchange site (P453L) and one at the FAD/pyridine nucleotide binding site (G194C); however, other mutations at the same locations failed to affect ROS generation. There were also no correlation between the changes in the physiological enzymatic activity of E3 and enhanced ROS production. Moreover, G194C mutation did not affect the enzymatic activity of E3 yet significantly stimulated ROS generation. It is interesting that this mutation is frequently associated with E3-deficiency common among Ashkenazi Jews (Shaag et al., 1999). Overall, the data imply that the ROS-generating activity of E3 could potentially be one of the important pathogenic factors in the clinical manifestation of E3-deficiencies associated with the mutations in Dld gene (Ambrus et al., 2011).

The H_2O_2 production by E3 was studied by Vinogradov's group on heart submitochondrial particles and isolated permeabilized heart mitochondria. Earlier, these researchers found that a fraction of matrix located proteins in bovine heart mitochondria can catalyze NADH-supported, ammonium-stimulated H_2O_2 production (Grivennikova et al., 2008). The key ROS producing enzyme in this fraction was later identified as dihydrolipoamide dehydrogenase (Kareyeva et al., 2012). Using alamethicin-permeabilized heart mitochondria, authors demonstrated that more than half of H_2O_2 production can be attributed to dihydrolipoamide dehydrogenase (alamethicin is a pore-forming peptide that is used in vitro to permeabilize mitochondria in order to gain unrestricted access to their matrix side. This allows precise experimental control of the pools of small molecules such as e.g. pyridine nucleotides or glutathione). In the presence of ammonium that stimulates dihydrolipoamide dehydrogenase activity most of the total H_2O_2 production (~90%) in alamethicin-permeabilized rat heart mitochondria was catalyzed by dihydrolipoamide dehydrogenase (Kareyeva et al., 2012). Recently, using a soluble form of Dld (pig heart DLDH obtained from Calzyme, U.S.A.) they also demonstrated that H_2O_2 production attributed to DLDH was dependent on the NAD^+/NADH ratio and exhibited first order dependence on O_2 concentration in the medium. Authors (Kareyeva et al., 2012) commented that the latter fact creates an apparent physiological paradox when the most ROS production by mitochondria is expected only upon significant reduction of their pyridine nucleotide pool whereas such a situation can arise only in deep hypoxia when ROS production is expected to be minimal due to its first-order nature of oxygen reactivity. This led the authors to predict that a burst of mitochondrial ROS production could be expected upon normoxic reperfusion of anoxic tissue because anaerobically deactivated complex I is only slowly acquiring its NADH oxidizing capacity upon the onset of normoxia (Maklashina et al., 2002).

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It should however be noted that oxygen accessibility to the oxygen reactive site in a protein-bound flavin (which is considered to be the site of ROS generation) in soluble DLDH may differ from that in a more physiologically relevant situation with enzyme complex-bound E3, e.g. in KGDHC or PDHC or other E3-bearing enzyme complexes. The binding itself is supposed to affect the conformation of the E3 protein, which is expected to affect the oxygen accessibility, the redox properties of the flavin and its reactivity with oxygen at its reactive site (4a position in the isoalloxazine ring (Kareyeva et al., 2012)). Nevertheless, these results suggest that E3-mediated ROS production could be of importance in oxidative stress and tissue damage upon ischemia and reperfusion. To note, ROS production by KGDHC is stimulated at lower (6.7–7.0) pH relevant to ischemia/reperfusion (Ambrus et al., 2009).

Concluding remarks

Although the fact of ROS generation by the E3 component of KGDHC is – in our opinion – well established, more work has to be done to demonstrate its pathophysiological role in oxidative stress at the level of organisms and to reveal the role of other E3-bearing enzymes in ROS production in different tissues. It would be of great interest to examine whether ROS-generation promoting point mutationsin E3 are present in brain tissue from humans affected by neurodegenerative diseases such as e.g., Alzheimer's or Parkinson's diseases. Hypothetically, such mutations do not necessarily have to be inherited; they can be acquired during the lifetime of an individual for example due to an exposure to pesticides or other environmental toxins. Another important goal is to establish whether the E3 enzyme is present in free, non-bound form in the matrix of mitochondria, and to study the rules and mechanisms governing its assembly into the enzyme complexes. It would also be of interest to evaluate the role of KGDHC (or other E3-bearing enzymes) in the oxidative stress associated with ischemia and reperfusion. Unfortunately, these studies at present are somewhat hindered by the absence of specific inhibitors acting at the E3 NAD^+ binding site, which is most likely the site of ROS production.

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