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ORIGINAL PAPER

Human 2-Oxoglutarate Dehydrogenase and 2-Oxoadipate Dehydrogenase Both Generate Superoxide/H_2O_2 in a Side Reaction and Each Could Contribute to Oxidative Stress in Mitochondria

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Abstract

According to recent findings, the human 2-oxoglutarate dehydrogenase complex (hOGDHc) could be an important source of the reactive oxygen species in the mitochondria and could contribute to mitochondrial abnormalities associated with multiple neurodegenerative diseases, including Alzheimer's disease, Huntington disease, and Parkinson's disease. The human 2-oxoadipate dehydrogenase (hE1a) is a novel protein, which is encoded by the *DHTKD1* gene. Both missence and nonsense mutations were identified in the *DHTKD1* that lead to alpha-aminoadipic and alpha-oxoadipic aciduria, a metabolic disorder with a wide variety of the neurological abnormalities, and Charcot-Marie-Tooth disease type 2Q, an inherited neurological disorder affecting the peripheral nervous system. Recently, the rare pathogenic mutations in *DHTKD1* and an increased H_2O_2 production were linked to the genetic ethiology of Eosinophilic Esophagitis (EoE), a chronic allergic inflammatory esophageal disorder. In view of the importance of hOGDHc in the tricarboxylic acid cycle (TCA cycle) and hE1a on the L-lysine, L-hydroxylysine and L-tryptophan degradation pathway in mitochondria, and to enhance our current understanding of the mechanism of superoxide/H_2O_2 generation by hOGDHc, and by human 2-oxoadipate dehydrogenase complex (hOADHc), this review focuses on several novel and unanticipated recent findings in vitro that emerged from the Jordan group's research. Most significantly, the hE1o and hE1a now join the hE3 as being able to generate the superoxide/H_2O_2 in mitochondria.

Keywords 2-Oxoglutarate and 2-oxoadipate dehydrogenase complexes · Thiamin diphosphate-enamine radical · Hydrogen peroxide · Oxidative stress

Abbreviations

hOGDHc	Human 2-oxoglutarate dehydrogenase complex
hE1o	2-Oxoglutarate dehydrogenase, the first E1 component of hOGDHc;
hE2o	Dihydrolipoyl succinyltransferase, the second E2 component of hOGDHc
hE3	Dihydrolipoyl dehydrogenase, the third E3 component of all 2-oxoacid dehydrogenase complexes
hOADHc	human 2-oxoadipate dehydrogenase complex, assembled from hE1a + hE2o + hE3
hE1a	2-Oxoadipate dehydrogenase, the first component of hOADHc
DHTKD1	Gene coding hE1a
TCA cycle	Tricarboxylic acid cycle
H_2O_2	Hydrogen peroxide
ROS	Reactive oxygen species
ThDP	Thiamin diphosphate
OG	2-Oxoglutarate
OA	2-Oxoadipate
DCPIP	2,6-Dichlorophenol-indophenol
EPR	Electron Paramagnetic Resonance.

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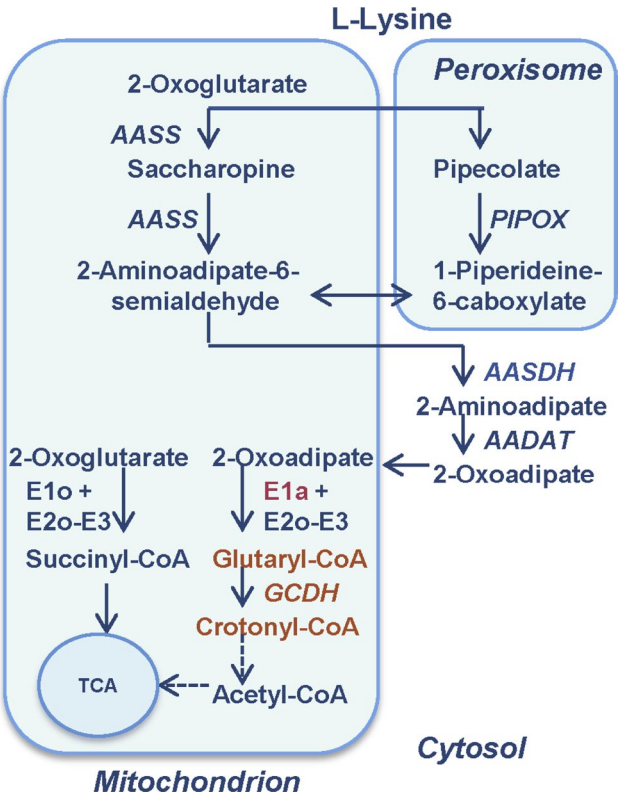
Introduction

Identification and characterization of enzymes utilizing the thiamin diphosphate (ThDP) cofactor and its enzyme-bound intermediates has been a special interest of the lead author's laboratory for decades [1–10]. As a continuation of this work, the group is currently focused on the catalytic mechanism, as well as on the assembly of the components in the human 2-oxoglutarate dehydrogenase multienzyme complex (hOGDHc, also known as the alpha-ketoglutarate dehydrogenase complex), a key regulatory multienzyme complex within the tricarboxylic acid cycle (TCA cycle) [9–11]. Recently, the group has also turned its attention to the novel 2-oxoadipate dehydrogenase complex (hOADHc), a related complex on the final degradation pathway of L-lysine, L-hydroxylysine and L-tryptophan [12, 13].

Diminished activities of the hOGDHc in brain, as well as reduced glucose metabolism, and the accompanying oxidative stress, have been correlated with multiple neurodegenerative diseases, including Alzheimer's disease, Huntington disease, and Parkinson's disease [14]. There have been several reports that mammalian OGDHc forms the reactive oxygen species (ROS) superoxide/H_2O_2 via reverse electron flow from NADH, which is regulated by the NADH/NAD^+ ratio [15–20]. In a comparison of the maximum capacities for superoxide /H_2O_2 production among 11 already known sites in skeletal muscle mitochondria, the rate of H_2O_2 production by OGDHc was approximately eight times higher than that from the flavin site (I_F) in the respiratory complex I, that is considered to be the main source of ROS generation in mitochondria [20, 21]. The findings suggested that the hOGDHc could be an important source of the reactive oxygen species in the mitochondria and could contribute to mitochondrial abnormalities associated with neurological disorders [14].

The human 2-oxoadipate dehydrogenase (hE1a) is a novel protein, which is encoded by the *DHTKD1* gene and is believed to be involved in the oxidative decarboxylation of 2-oxoadipate to glutaryl-CoA on the L-lysine degradation pathway (Scheme 1). Both missence and nonsense pathogenic mutations were identified in the *DHTKD1* gene that lead to alpha-aminoadipic and alpha-ketoadipic aciduria (AMOXAD: MIM 204750), a metabolic disorder with a wide variety of neurological abnormalities. Those range from mild to severe intellectual disability, muscular hypotonia, developmental delay, ataxia and epilepsy, that have been described in the newborn and in children at age of 7–14 years [22–24]. It was also reported that nonsense mutations in the *DHTKD1* gene cause Charcot-Marie-Tooth disease type 2Q (CMT 2Q: MIM 615025), an inherited neurological disorder affecting the peripheral nervous system [25–27]. Recent findings demonstrated that *DHTKD1* deficiency causes a



Scheme 1 The L-lysine degradation pathways in the cytosol and in mitochondria. *AASS*, 2-aminoadipate-6-semialdehyde synthase; *PIPOX*, pipecolate oxidase; *AASDH*, 2-aminoadipate-6-semialdehyde dehydrogenase; *AADAT*, 2-aminoadipate transaminase; E1o, E2o, E3-components of the hOGDHc; E1a,-2-oxoadipate dehydrogenase; *GCDH*, glutaryl-CoA dehydrogenase (Scheme 1 was modified from Ref. [22])

CMT2Q-like phenotype in mice and leads to accumulation of alpha-ketoadipic acid and alpha-aminoadipic acid in urine that could contribute to the pathogenesis of CMT2Q [28]. Recently, rare pathogenic mutations in the *DHTKD1* and *OGDHL* genes (*OGDHL*, oxoglutarate dehydrogenase-like homologue) were identified by whole-exome sequencing, implicating the *DHTKD1* and *OGDHL* pathogenic mutations in the genetic etiology of Eosinophilic Esophagitis (EoE), a chronic allergic inflammatory esophageal disorder [29]. According to data reported in the literature, the *DHTKD1* silencing impaired the mitochondrial function in hepatic carcinoma (HeG2) cell line [26], esophageal epithelial cell line (EPC2) and patient fibroblasts with Eosinophilic Esophagitis [29]. It was reported that *DHTKD1* knockout could be correlated with reduced ATP production, impaired mitochonrial biogenesis and increased H_2O_2 production, suggesting that *DHTKD1* contributes to mitochondrial function maintenance [26, 29]. These findings from the two groups provide direct evidence for an association between an impaired *DHTKD1* gene expression and mitochondrial disfunction.

Pharmacological inhibition of the *DHTKD1 gene* encoded hE1a could be a promising strategy by using the substrate reduction therapy approach to treat glutaric aciduria type I (GA I: MIM 231670), a metabolic disorder characterized by a progressive movement disorder that begins during the first year of life and is caused by mutations in the *GCDH* gene encoding glutaryl-CoA dehydrogenase (GCDH). The GCDH is located downstream of hE1a on the L-lysine degradation pathway (Scheme 1) [30]. Deficiency of GCDH is accompanied by elevated concentrations of glutaryl-CoA, neurotoxic glutaric acid and 3- hydroxyglutaric acid, and nontoxic glutarylcarnitine in body tissues and body fluids, particularly within the brain, with a risk to develop encephalopatic crises and striatal damage [31, 32]. However, the molecular mechanisms leading to pathogenesis of GA I are poorly understood, as is the function of the hOADHc. It was suggested that glutaryl-CoA formed by hOADHc could serve as a substrate for post-translational modification of the mitochondrial proteins by lysine glutarylation, which could be a pathogenic mechanism underlying GA I [33, 34]. However, recent findings from the Jordan's group suggest that pharmacological inhibition solely of the hE1a protein is insufficient as a therapeutic approach [10].

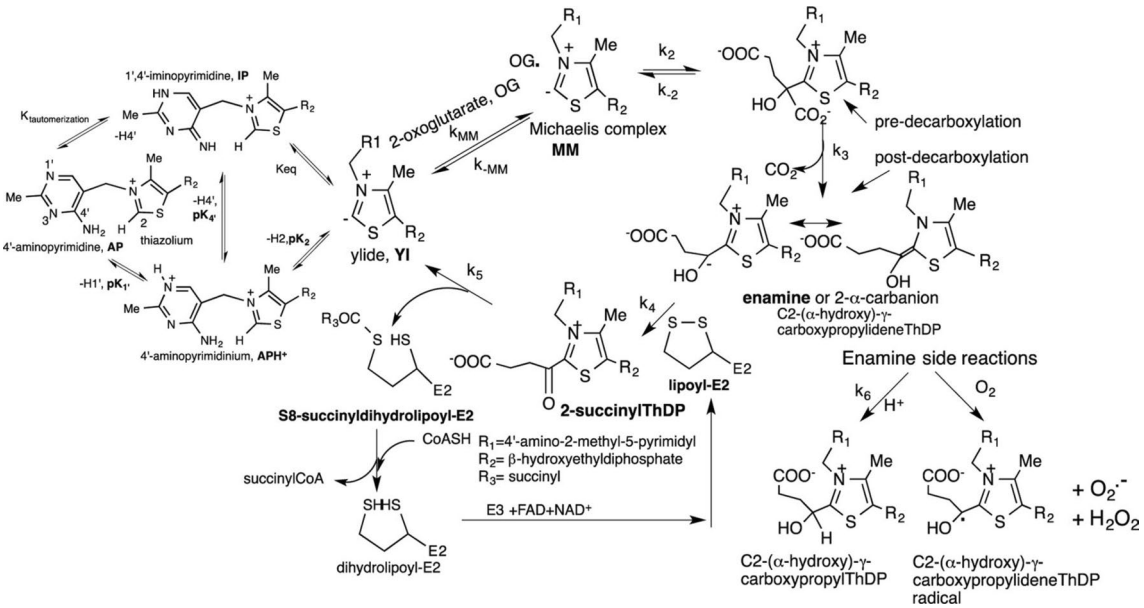
In view of the importance of hOGDHc and hOADHc in mitochondrial metabolic pathways, and in neurological development, and to enhance our current understanding of the mechanism of superoxide /H_2O_2 generation by hOGDHc and by hOADHc, this review focuses on several novel and unanticipated recent findings in vitro that emerged from the authors' research.

The E1o Component of the 2-Oxoglutarate Dehydrogenase Complex Forms a Thiamin Diphosphate-Derived Radical by Aerobic Oxidation of the ThDP-Enamine Intermediate

The hOGDHc catalyzes the conversion of 2-oxoglutarate to succinyl-CoA according to the overall reaction (Eq. 1, Scheme 2) and represents a key regulatory enzyme within the TCA cycle, controlling the reduction potential (NADH/NAD^+) of the mitochondria and ATP synthesis [35–40].

$$2-\text{oxoglutarate} + NAD^+ + CoA \rightarrow \text{succinyl}-CoA + NADH + H^+ + CO_2 \quad (1)$$

The hOGDHc (M_r of 5×10^6) consists of multiple copies of three catalytic components: a ThDP-dependent 2-oxoglutarate dehydrogenase (hE1o, α_2 homodimer, EC 1.2.4.2; 115,935 Da/monomer) that catalyzes the decarboxylation of 2-oxoglutarate and the subsequent reductive succinylation of the lipoyl group covalently amidated to the dihydrolipoamide succinyltransferase component [hE2o octahedral (24-mer), EC 2.3.1.61, 48,755 Da/monomer] (Scheme 2). The succinyl



Scheme 2 Mechanism of hOGDHc with alternative fates of the enamine [9]. The ThDP-dependent hE1o catalyzes the decarboxylation of 2-oxoglutarate, releasing CO_2, followed by the reductive succinylation of the hE2o carrying a covalently amidated lipoic acid as a redox cofactor. The reductive succinylation reaction is followed by trans-thiolesterification of the acyl group onto CoA in the active centers of hE2o leading to production of succinyl-CoA. The hE3 with a tightly, but non-covalently bound FAD as cofactor re-oxidizes dihydrolipoamide on hE2o with the reduction of NAD^+ to NADH

group is then transferred from succinyl dihydrolipoamide-E2o to CoA with the formation of succinyl-CoA in the active center of hE2o. Next, the dihydrolipoamide-E2o is reoxidized by FAD non-covalently bound to dihydrolipoamide dehydrogenase (hE3, homodimer, EC 1.8.1.4, 55,177 Da/monomer). Finally, the $FADH_2$ reduces NAD^+ to NADH ($+H^+$) (Scheme 2). The E3 component is common to all of the human 2-oxoacid dehydrogenase complexes, such as pyruvate dehydrogenase (PDHc), OGDHc, branched-chain 2-oxoacid dehydrogenase (BCOADH) and, the recently added OADHc. The succesful expression and purification of functionally active, full-length recombinant hE1o, hE2o, and hE3 components of the hOGDHc enabled us to investigate the redox chemistry on hOGDHc in studies performed in vitro leading to novel findings that we believe make a significant contribution to our understanding of an important topic of interest, the efficiency of superoxide/H_2O_2 production by hOGDHc. Below, we have summarized our recent findings in vitro on the hOGDHc [9–13].

Detection of the ThDP-Derived Radical Species on E1o by EPR

Generation of the ThDP-derived radical species, the C2-(α-hydroxy)-γ-carboxypropylidene ThDP radical (hence forth called the ThDP-enamine radical), by the reaction of hE1o with ThDP and 2-oxoglutarate (OG) aerobically was detected by electron paramagnetic resonance (EPR) [9].

Formation of the ThDP-enamine radical is one of three side reactions of the hE1o-ThDP-enamine intermediate depicted in the lower right quadrant of Scheme 2. The half-life of the ThDP-enamine radical species was estimated at ~ 1 min [10]. Importantly, the radical species were detected at different times of incubation with 2-oxoglutarate, although the X-band EPR spectra displayed lower amplitude at longer times of incubation (Fig. 1) [10]. These findings are consistent with those reported earlier on the *E. coli* E1o (ecE1o), where the ThDP-derived radical species had been detected by EPR on aerobic oxidation of 2-oxoglutarate by ecE1o in an off-pathway reaction [41]. Next, generation of the ThDP-enamine radical species was also detected by EPR upon assembly of the hE1o, hE2o and hE3 components into hOGDHc, indicating that assembly to hOGDHc does not affect formation of the ThDP-enamine radical species on the hE1o. Spin quantification measurements enabled determination of the amount of ThDP-derived radical species to be ~ 0.9 μM for the hE1o-ThDP-2-oxoglutarate system (0.2% occupancy of the hE1o active centers) and ~ 1.3 μM for the hE1o assembled into hOGDHc (0.59% occupancy of the hE1o active centers) [9]. It needs to be noted that while the resolution of the observed X-band continuous wave EPR spectra did not allow us to provide a detailed assignment of the protons contributing to the spectrum, the principal g-values used in the simulation are those measured for the ThDP-enamine radical species (Fig. 1) [42]. For a more rigorous assignment of g-values, future experiments using high frequency EPR, isotopic substitutions, electron nuclear double resonance, and /or electron spin echo envelope modulations will have to be carried out.

The ThDP-Enamine Intermediate Precedes the ThDP-Enamine Radical on E1o

Circular dichroism, UV–visible spectroscopy and ^{1}H NMR studies provide the cumulative evidence for the existence of a stable ThDP-enamine intermediate (the first ThDP-bound post-decarboxylation intermediate) preceding the ThDP-enamine radical on both hE1o and ecE1o (see Scheme 2, upper right quadrant) [9]. It was suggested that the stability of the ThDP-enamine intermediate is likely due to some stabilization afforded by the side chain C5 carboxylate group (see Scheme 3); perhaps via an intramolecular hydrogen bond formed with the enaminolate oxyanion, or of the lone pair of electrons at C2α in the enamine forming an intramolecular hydrogen bond.

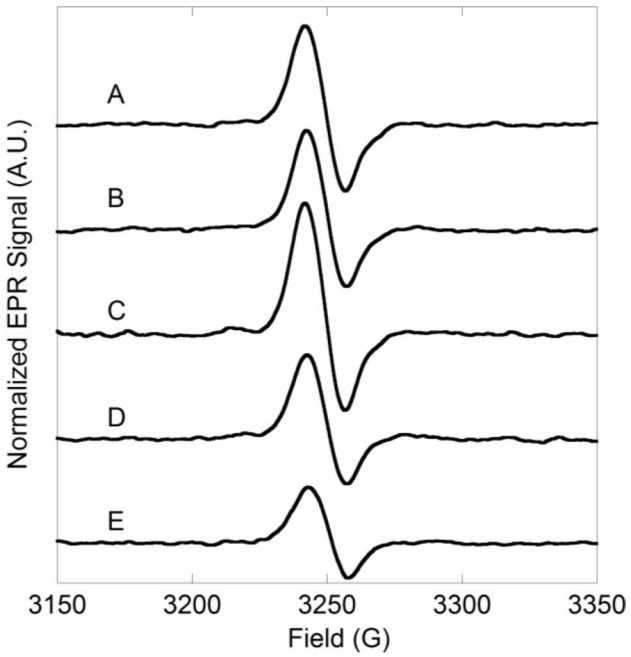
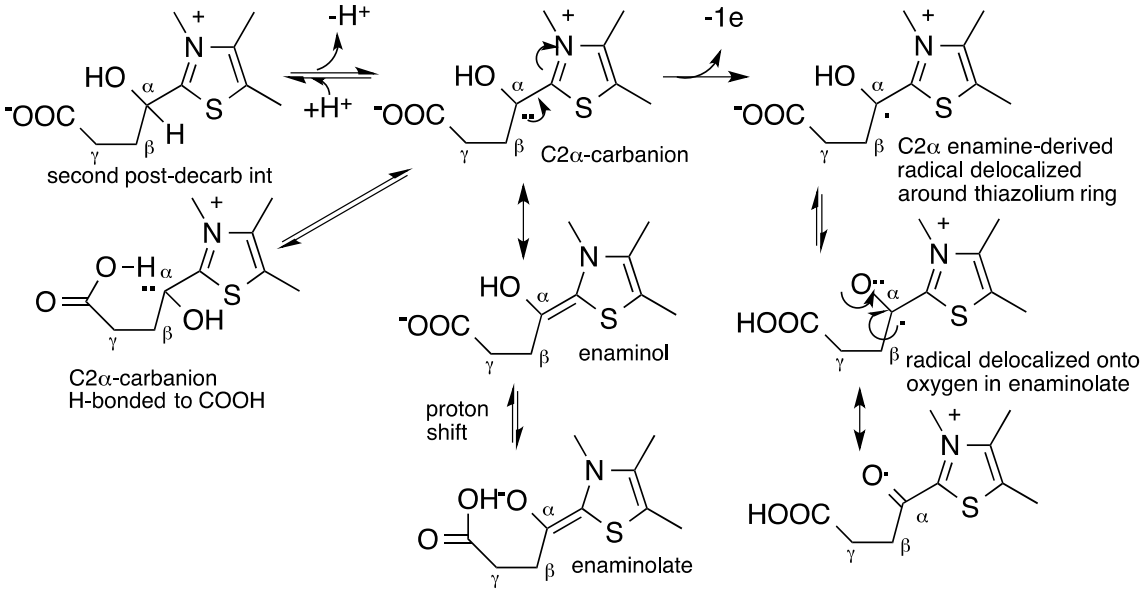


Fig. 1 X-band EPR spectra of the ThDP-enamine radical species generated on hE1o from 2-oxoglutarate [10]. Stack-plot of EPR spectra generated at different times of incubation of hE1a (0.275 mM concentration of active centers) with 0.5 mM ThDP and 10 mM 2-oxoglutarate aerobically: 28 s (A); 35 s (B); 40 s (C); 76 s (D); 180 s (E)



Scheme 3 Possible role of side chain carboxylate in stabilizing the ThDP-bound enamine and ThDP-enamine radical [9]

Production of Superoxide/H_2O_2 by hE1o

Production of superoxide by hE1o was directly detected by the cyt *c* assay in collaboration with Professor Adam-Vizi's group at Semmelweis University (Budapest, Hungary) [9, 43]. An hE1o-specific activity for superoxide generation of 2.06 ± 0.07 nmol min^{-1} mg $hE1o^{-1}$ was determined, comprising ~0.12% of the hE1o-specific activity measured in the assay containing a redox dye 2,6-dichlorophenolindophenol (DCPIP) as an artificial electron acceptor. The generation of H_2O_2 by the hE1o component was detected in the Amplex Red fluorescence assay, a widely used approach [44]. An hE1o specific activity of 4.05 ± 0.10 nmol min^{-1} mg.

$hE1o^{-1}$ was determined for H_2O_2 generation with a capacity of ~0.23% of the hE1o specific assay, which is certainly significant. The primary product is proposed to be superoxide, resulting from a one-electron oxidation of the ThDP-enamine intermediate by molecular oxygen (see Scheme 2, lower right quadrant); the H_2O_2 present in the assay could be considered to be the product of spontaneous dismutation of superoxide, rather than a two-electron reduction of molecular oxygen to H_2O_2 [9].

Generation of the Reactive Oxygen Species by hE1o from 2-Oxoadipate

We also demonstrated by studies in vitro that hE1o, and hOGDHc assembled from individual components, could both generate the ThDP-enamine radical species and H_2O_2 from 2-oxoadipate (OA), the next higher homologue of 2-oxoglutarate, differing from it by an additional CH_2 group [10]. The EPR spectra of the ThDP-enamine radical species generated on hE1o from 2-oxoglutarate, and from 2-oxoadipate under similar conditions revealed that the radical species generated from 2-oxoadipate is approximately 3 times lower in concentration and the spectrum has a slightly broader line width than that generated from 2-oxoglutarate (Fig. 2), making determination of the absolute concentration of radical species difficult [10]. H_2O_2 activities of 1.958 nmol min^{-1} mg $hE1o^{-1}$ (in the absence of ADP) and 4.093 nmol·min^{-1}·mg $hE1o^{-1}$ (in the presence of 0.4 mM ADP) were determined, and were comparable to that formed with 2-oxoglutarate, or even greater under similar conditions [10].

There is some controversy in the literature regarding superoxide/H_2O_2 production by hOGDHc from 2-oxoadipate. Our findings summarized above suggest that the hE1o component could be an efficient source of superoxide/H_2O_2 generation in mitochondria from 2-oxoadipate, and could at least partially compensate for the loss of *DHTKD1* function as had been suggested by others [30]. An alternative viewpoint suggested that superoxide/H_2O_2 generation from 2-oxoadipate be solely assigned to the OADHc, particularly to the E3-bound FAD, while the contribution from the OGDHc was considered as insignificant [45, 46]. To differentiate the contribution from hOGDHc and hOADHc to superoxide/H_2O_2 generation, the synthesis of enzyme-specific inhibitors and their analysis in vitro and on isolated mitochondria would be required. Identification of such inhibitors could also help to develop pharmacological inhibitors of the hE1a to treat glutaric aciduria type I,

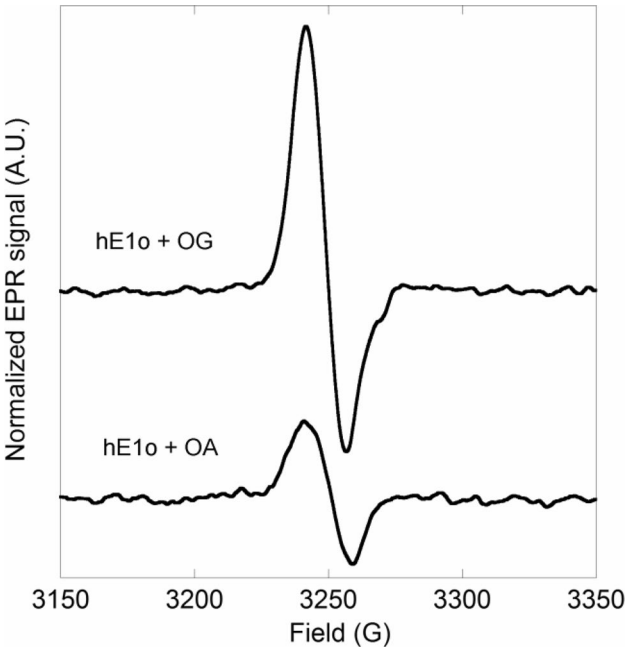


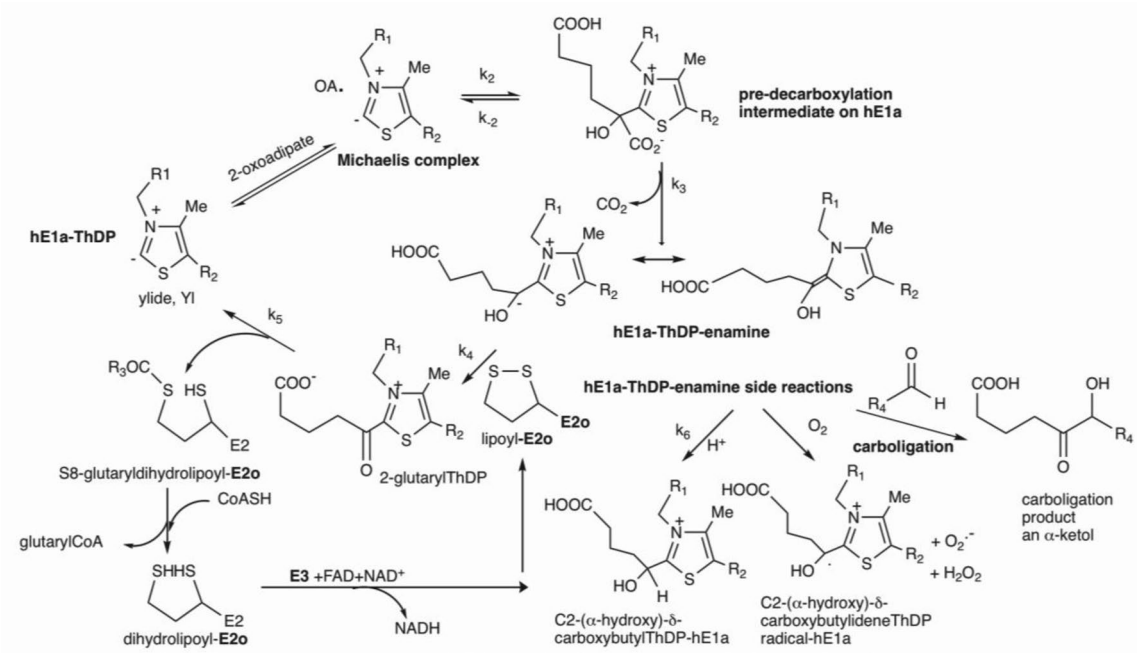
Fig. 2 Comparison of the X-band EPR spectra of the ThDP-enamine radical species generated on hE1o from 2-oxoglutarate and from 2-oxoadipate [10]. (Top) EPR spectra of the ThDP-enamine radical species generated on hE1o from 2-oxoglutarate. The hE1o (0.225 mM concentration of active centers) in 50 mM HEPES (pH 7.5) containing 0.50 mM ThDP, 2.0 mM $MgCl_2$ and 0.15 M NaCl was mixed with 10 mM 2-oxoglutarate at room temperature. (Bottom) EPR spectra of the ThDP-enamine radical species generated on hE1o from 2-oxoadipate. The hE1o (0.268 mM concentration of active centers) was mixed with 10 mM 2-oxoadipate in the same buffer sysytem. Both samples were flash-frozen in liquid nitrogen after 40 s of incubation

a metabolic disorder of the L-lysine, L-hydroxylysine and L-tryptophan degradative pathway [30, 34, 47]. This issue became even more complicated in light of our recent findings in vitro suggesting that the hE1o and hE1a components could each assemble with the same hE2o and hE3 components into the corresponding complexes, and that they both could generate superoxide/H_2O_2 from 2-oxoglutarate and 2-oxoadipate [12].

What Have we Learnt About the Human 2-Oxoadipate Dehydrogenase Complex and its Contribution to Superoxide/H_2O_2 Generation from In Vitro Studies?

It had long been assumed, that similarly to the mitochondrial hOGDHc in the TCA cycle, the ThDP-dependent 2-oxoadipate dehydrogenase (hE1a, probably an α_2 homodimer; EC: 1.2.4.2; 103,077 Da/monomer) is the first component of the hOADHc, which, along with its specific dihydrolipoamide glutaryltransferase (hE2a, the second component of the hOADHc, the gene localization is not identified to date), and the hE3 carry out the principal reactions for glutaryl-CoA formation according to the overall reaction in Eq. 2 and detailed chemistry in Eqs. 3–6 and in Scheme 4:

$$2-\text{oxoadipate} + \text{NAD}^+ + \text{CoA} \rightarrow \text{glutaryl}-\text{CoA} + \text{NADH} + \text{H}^+ + \text{CO}_2 \quad (2)$$



Scheme 4 Proposed mechanism for the hE1a, hE2o and hE3 components on assembly into hOADHc [13]

$$2-\text{Oxoadipate}+\text{hE1a}\rightarrow \text{C2}-(\alpha-\text{hydroxy})-\delta-\text{carboxybutylidene}-\text{ThDP}-\text{hE1a(hE1a}-\text{ThDP}-\text{enamine intermediate})+\text{CO}_2 \quad (3)$$

$$\text{C2}-(\alpha-\text{hydroxy})-\delta-\text{carboxybutylidene}-\text{ThDP}-\text{hE1a}+\text{lipoyl}-\text{hE2oS8}-\text{glutaryldihydrolipoyl}-\text{hE2o(reductive glutarylation)} \quad (4)$$

$$\text{S8}-\text{glutaryldihydrolipoyl}-\text{hE2o}+\text{CoA}\rightarrow \text{glutaryl}-\text{CoA}+\text{dihydrolipoyl}-\text{hE2o} \quad (5)$$

$$\text{dihydrolipoyl}-\text{hE2o}+\text{hE3}+\text{NAD}^+\rightarrow \text{lipoyl}-\text{hE2o}+\text{NADH} \quad (6)$$

However, there is no evidence to date for the existence of a specific hE2a that would suggest that the hE1a is the first component of a novel hOADHc, nor is there evidence for the existence of an OGDH-like hybrid complex with function via the TCA cycle. In view of the importance of the hOADHc in neuropathogenesis of metabolic disorders, the *DHTKD1* gene was synthesized and functionally active hE1a protein was expressed for the first time, as judged according to kinetic and spectroscopic criteria available in the Jordan group for these 2-oxo acid dehydrogenase multienzyme complexes. Our studies in vitro presented below revealed several novel findings [12, 13].

Different Substrate Preferences Have Been Displayed by hOGDHc and hOADHc

The hOGDHc and hOADHc, while they share the same hE2o and hE3, and follow a similar mechanism (see Scheme 2 for hOGDHc and Scheme 4 for hOADHc), displayed different substrate preferences in accord with their functional role in the TCA cycle, and on the L-lysine degradation pathway [12]. From the data presented in Table 1, it is evident that hOGDHc (hE1o + hE2o + hE3) has preference for 2-oxoglutarate over 2-oxoadipate as substrate [compare the values of the catalytic efficiency (k_{cat}/K_m) in Table 1 for the two substrates]. In comparison, the hOADHc (hE1a + hE2o + hE3) displayed preference for 2-oxoadipate $k_{cat}/K_{m,OA} = 398\times10^3\ \text{M}^{-1}\text{s}^{-1}$ over 2-oxoglutarate ($k_{cat}/K_{m,OG} = 8.1\times10^3\ \text{M}^{-1}\text{s}^{-1}$).

A comparison of the two complexes revealed that hOADHc is about 40-fold more efficient with 2-oxoadipate compared to hOGDHc, that could be partially explained by its low $K_{m,OA}$ value of 0.015 mM [13]. Taking into account the $K_{m,OG}$ of 0.15 mM and the $K_{m,OA}$ of 0.52 mM, both determined in the overall hOGDHc reaction, the findings cannot exclude the possibility that the hOGDHc could function as a 2-oxoadipate dehydrogenase, particularly when the *DHTKD1* function is suppressed and the concentration of

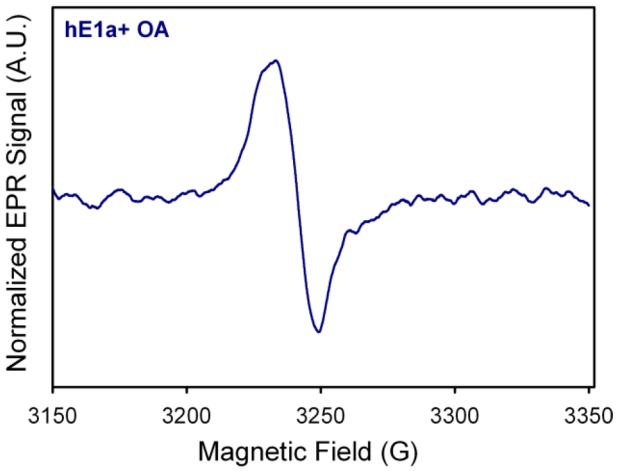


Fig. 3 X-band EPR spectrum of the ThDP-enamine radical species observed on hE1a on addition of 2-oxoadipate [13]. The hE1a (0.418 mM concentration of the active centers) in 0.1 M Tris·HCl (pH 7.5) containing 0.50 mM ThDP and 2.0 mM $MgCl_2$ was mixed with 10 mM 2-oxoadipate aerobically. The mixture was immediately transferred into an EPR tube and was flash-frozen in liquid nitrogen after ~30–40 s of incubation with 2-oxoadipate

Table 1 Kinetic parameters for the hOGDHc and hOADHc assembled in vitro [12]

Enzyme	Overall activity 2-oxoglutarate (u/mg hE1)[a]	$K_{m,OG}$ (mM)	$k_{cat}/K_{m,OG}$ ($\text{M}^{-1}\ \text{s}^{-1}$)	Overall activity 2-oxoadipate (u/mg E1)[a]	$K_{m,OA}$ (mM)	$k_{cat}/K_{m,OA}$ ($\text{M}^{-1}\ \text{s}^{-1}$)
hOGDHc[b]	5.63 ± 0.59	0.15	142×10^3	1.38 ± 0.07	0.52	10×10^3
hOADHc[c]	0.58 ± 0.04	0.25	8.1×10^3	1.72 ± 0.07	0.015	398×10^3

[a]One unit of activity is defined as the amount of NADH produced (μmol·min^{-1} mg hE1^{-1})

[b]For the hOGDHc assembly, hE1o (0.125 mg) was mixed with hE2o (0.250 mg) and hE3 (0.125 mg)

[c]For the hOADHc assembly, hE1a (0.4 mg) was mixed with hE2o (0.8 mg) and hE3 (2.0 mg)

Table 2 Comparison of the catalytic efficiency of hE1o and hE1a in the reaction leading to H_2O_2 production from 2-oxoadipate [10, 13]

Enzyme	H_2O_2 activity (nmol·min^{-1}·mg hE1^{-1})	k_{cat} (s^{-1})	$k_{cat}/K_{m,OA}$ ($M^{-1}s^{-1}$)
hE1o	1.958 ± 0.099[a]	0.0074[a]	0.027×10^3
	4.093 ± 0.445[b]	0.016[b]	0.059×10^3
hE1o + hE2o + hE3	2.668 ± 0.188[a]	0.010[a]	0.019×10^3
	2.086 ± 0.126[b]	0.008[b]	0.015×10^3
hE1a	6.94 ± 0.99	0.024	6.5×10^3
hE1a + hE2o + hE3	(4.7–10.5)	0.026	(4–10)x10^3

[a]The H_2O_2 activity was measured in the absence of ADP

[b]The H_2O_2 activity was measured with 0.40 mM ADP added

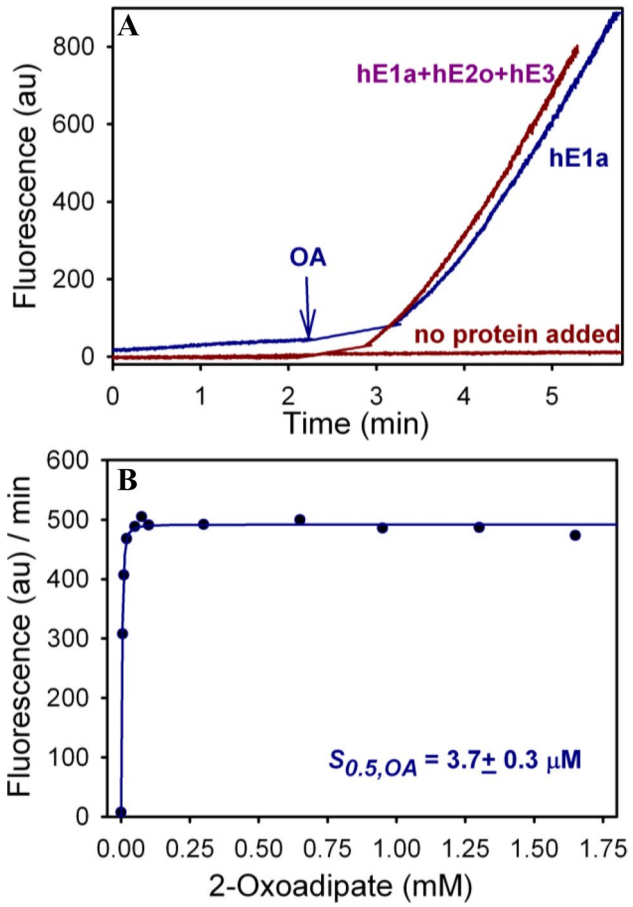


Fig. 4 Progress curves of H_2O_2 production by hE1a from 2-oxoadipate [13]. **a** Progress curves of H_2O_2 production by hE1a by itself and by hE1a assembled with hE2o and hE3 components into hOADHc. **b** Dependence of the H_2O_2 production by hE1a on concentration of 2-oxoadipate

the 2-oxoadipate substrate is elevated. The kinetic findings in vitro, however, could not answer the question whether substrate channeling in hOGDHc could be affected by sharing the hE2o component with hE1a on the L-lysine degradation pathway. Further studies with isolated mitochondria and cell lines, as well as the synthesis of hE1o and hE1a specific inhibitors is required, and are planned for future studies.

Formation of the Reactive Oxygen Species by hE1a from 2-Oxoadipate

The X-band EPR spectra of the hE1a incubated with 2-oxoadipate aerobically revealed formation of the ThDP-enamine radical species on hE1a (Fig. 3). The concentration of ThDP-enamine radical species in the hE1a active centers was estimated as 0.2 µM (0.1% occupancy) and 0.5 µM (0.12% occupancy) in two independent experiments when derived from 2-oxoadipate; and even less (< 0.1 µM) was generated from 2-oxoglutarate [12, 13]. The H_2O_2 activities and the calculated values of k_{cat} were not very different for hOGDHc and hOADHc with 2-oxoadipate as substrate. However, the catalytic efficiency of the hOADHc in this reaction was ~(235–588)-fold higher compared to hOGDHc, again due to its low $K_{m,OA}$ value. These findings suggest that on elevation of the 2-oxoadipate level, the hOGDHc could function as 2-oxoadipate dehydrogenase and could contribute to superoxide/H_2O_2 production [12, 13] (Table 2).

Potential Cross-Talk Between the TCA cycle and the L-Lysine Degradation Metabolic Pathways

Our studies in vitro demonstrated that the hE1a and hE1o experienced strikingly different regulation by the glutaryl-CoA and succinyl-CoA products. The activity of the hOGDHc, but not of the hOADHc, was significantly reduced by both succinyl-CoA and glutaryl-CoA, indicating that it is indeed the first component (hE1a, hE1o) of the corresponding complexes that is subject to regulation rather than hE2o and hE3 (Fig. 4) [12]. We hypothesized that the hOADHc could also function as a donor of glutaryl groups for reversible post-translational modification of the proteins by lysine glutarylation, a new type of lysine modification

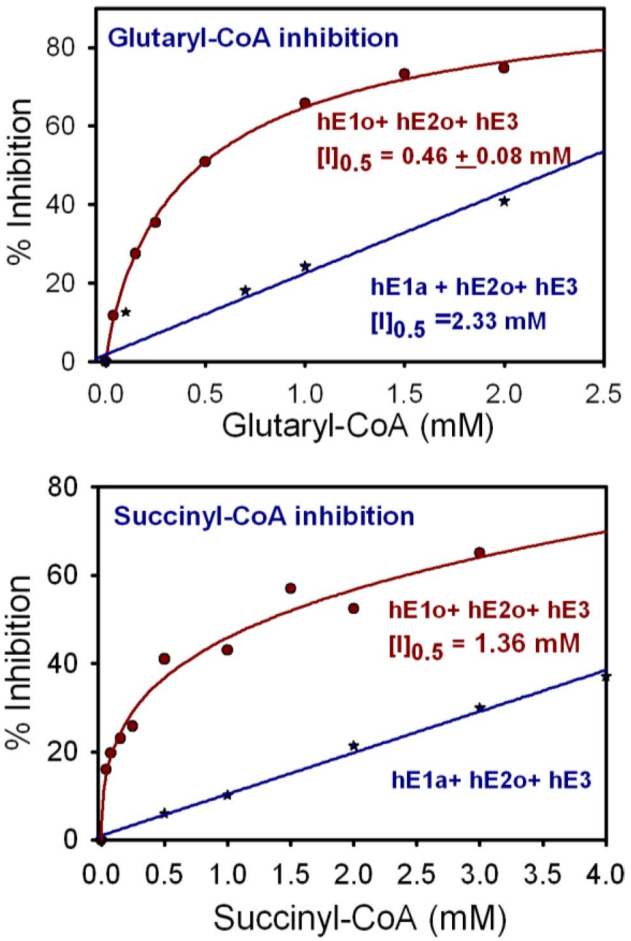


Fig. 5 Inhibition of NADH production by glutaryl-CoA (top) and by succinyl-CoA (bottom) of hE1o and hE1a assembled to hOGDHc and hOADHc with the same hE2o and hE3 components [13]

only recently reported in the literature that could be relevant in some disease states, such as glutaric aciduria I [33, 34]. Using mass-spectrometry based proteomic screening carried out on lysine glutarylation in mouse liver, 683 lysine glutarylated sites in 191 proteins were identified with predominant glutarylation of the metabolic enzymes and of mitochondrial proteins [33, 34]. Our studies in vitro suggest that hE2o can serve as a trans-glutarylase, in addition to being a trans-succinylase, a function suggested by Gibson's group [48]. We will address these possibilities in future studies (Fig. 5).

Conclusions and Future Perspective

In this review we compared and contrasted the potential aerobic oxidative chemistry of the hOGDHc and hOADHc, chemistry that could be implicated in human pathology. The hE1o and hE1a components, while they share the same hE2o and hE3 and follow a similar mechanism, displayed different substrate preferences and different regulation by their acyl-coenzyme A products, consistent with their participation on two metabolic pathways. Related to their contribution to superoxide/H_2O_2 generation we can conclude that:

(i) The hE1o and hE1a incubated aerobically with 2-oxoglutarate and 2-oxoadipate revealed the formation of the ThDP-enamine radical species. The O_2-induced oxidation of the ThDP-enamine intermediate with formation of the ThDP-enamine radical species is "off-pathway" and represents less than 1% of the "on-pathway" reactivity of the ThDP-enamine intermediate toward reductive succinylation /glutarylation of hE2o and succinyl-CoA/glutaryl-CoA formation.

(ii) The findings suggest that the "on-pathway" mechanism produces succinyl-CoA/glutaryl-CoA via 2-electron oxidation of the ThDP-enamine intermediate (ionic mechanism) proposed earlier in a model reaction [49].

(iii) The hE1o, and the hOGDHc assembled from individual components, could both produce superoxide/ H_2O_2 from both 2-oxoglutarate and 2-oxoadipate. Taking into account similar values of $K_{m,OG}$ (0.15 mM) and of $K_{m,OA}$ (0.52 mM), both determined in the overall hOGDHc reaction, the findings in vitro suggest that the hOGDHc could at least partially compensate for the loss of *DHTKD1* function.

(iv) The catalytic efficiency of the hOADHc in the reaction leading to H_2O_2 production from 2-oxoadipate was approximately (235–588)-fold greater than that produced by hOGDHc, mainly due to its low $K_{m,OA}$ value.

(v) Most importantly, the hE1o and hE1a now join the hE3 as being able to generate the superoxide/H_2O_2 in mitochondria. The results with the hE1a further support our first report of the formation of the ThDP-enamine radical by the E1 components under aerobic conditions [9].

In our future studies we wish to address the folowing issues: (i) How is the ThDP-enamine radical stabilized in the active centers of hE1a and hE1o. (ii) Do the pathogenic mutations identified in the *DHTKD1* gene affect the H_2O_2 production by hOADHc. (iii) Could we differentiate in mitochondria the contribution of hOGDHc and hOADHc to superoxide/H_2O_2 generation. (iv) We also wish to determine how acidic glutarylation of hE1o and/or hE1a lysines by glutaryl-CoA influences their structure and function. This would be important for understanding the potential cross-talk between the TCA cycle and L-lysine degradation metabolic pathways and to understand the physiological role of lysine glutarylation in post-translational modification of proteins.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest to declare.

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