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Original article

The human Krebs cycle 2-oxoglutarate dehydrogenase complex creates an additional source of superoxide/hydrogen peroxide from 2-oxoadipate as alternative substrate

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ABSTRACT

Recently, we reported that the human 2-oxoglutarate dehydrogenase (hE1o) component of the 2-oxoglutarate dehydrogenase complex (OGDHc) could produce the reactive oxygen species superoxide and hydrogen peroxide (detected by chemical means) from its substrate 2-oxoglutarate (OG), most likely concurrently with one-electron oxidation by dioxygen of the thiamin diphosphate (ThDP)-derived enamine intermediate to a C2α-centered radical (detected by Electron Paramagnetic Resonance) [Nemeria et al., 2014 [17]; Ambrus et al. 2015 [18]]. We here report that hE1o can also utilize the next higher homologue of OG, 2-oxoadipate (OA) as a substrate according to multiple criteria in our toolbox: (i) Both E1o-specific and overall complex activities (NADH production) were detected using OA as a substrate; (ii) Two post-decarboxylation intermediates were formed by hE1o from OA, the ThDP-enamine and the C2α-hydroxyalkyl-ThDP, with nearly identical rates for OG and OA; (iii) Both OG and OA could reductively acylate lipoyl domain created from dihydrolipoyl succinyltransferase (E2o); (iv) Both OG and OA gave α-ketol carboligaton products with glyoxylate, but with opposite chirality; a finding that could be of utility in chiral synthesis; (v) Dioxygen could oxidize the ThDP-derived enamine from both OG and OA, leading to ThDP-enamine radical and generation of superoxide and H_2O_2. While the observed oxidation-reduction with dioxygen is only a side reaction of the predominant physiological product glutaryl-CoA, the efficiency of superoxide/ H_2O_2 production was 7-times larger from OA than from OG, making the reaction of OGDHc with OA one of the important superoxide/ H_2O_2 producers among 2-oxo acid dehydrogenase complexes in mitochondria.

1. Introduction

The human 2-oxoglutarate dehydrogenase multienzyme complex (hOGDHc) is a key enzyme in the tricarboxylic acid (TCA) cycle and consists of multiple copies of three enzymes (Scheme 1): a thiamin diphosphate (ThDP)-dependent 2-oxoglutarate dehydrogenase (hE1o; EC1.2.4.2), dihydrolipoyl succinyl transferase (hE2o; EC 2.3.1.61), and dihydrolipoyl dehydrogenase (hE3; EC1.8.1.4) (Scheme 1) [1–4]. A diminished activity of hOGDHc has been linked to numerous neurodegenerative diseases, such as Alzheimer's disease [5,6], Parkinson disease [7], Huntington's disease [8,9], and progressive supranuclear palsy [10]. Recently, it was reported that OGDHc is involved in cellular regulation of mitochondrial proteins via succinylation of lysine residues [11] and the gene encoding OGDHc was identified as being responsible for stabilization of the hypoxia-inducible transcription factors (HIF's) under aerobic conditions [12]. Much attention has been focused on the generation of the reactive oxygen species (superoxide/H_2O_2), by hOGDHc [13–21]. It is known that hOGDHc has the highest capacity of H_2O_2 generation within the NADH/NAD^+ isopotential group in mammalian mitochondria, which also includes the pyruvate dehydrogenase complex (PDC), branched-chain α-ketoacid dehydrogenase complex (BCKDHc), and more recently, the putative 2-oxoadipate

Abbreviations: hE1o, human 2-oxoglutarate dehydrogenase; hE2o, human dihydrolipoyl succinyltransferase; hE3, human dihydrolipoyl dehydrogenase; hOGDHc, human 2-oxoglutarate dehydrogenase complex; PDC, pyruvate dehydrogenase complex; ecE1o, *E. coli* 2-oxoglutarate dehydrogenase; ecE2o, *E. coli* dihydrolipoyl succinyltransferase; DHTKD1, gene coding for dehydrogenase E1 and transketolase domain-containing protein 1; HRP, horseradish peroxidase; SOD, superoxide dismutase; ThDP, thiamin diphosphate; DCPIP, 2,6-dichlorophenol-indophenol; OG, 2-oxoglutarate; OA, 2-oxoadipate; hLDo, human lipoyl domain of hE2o; KMV, α-keto-β-methyl-*n*-valeric acid; FT-MS, Fourier transform mass spectrometry; CD, circular dichroism; EPR, Electron Paramagnetic Resonance

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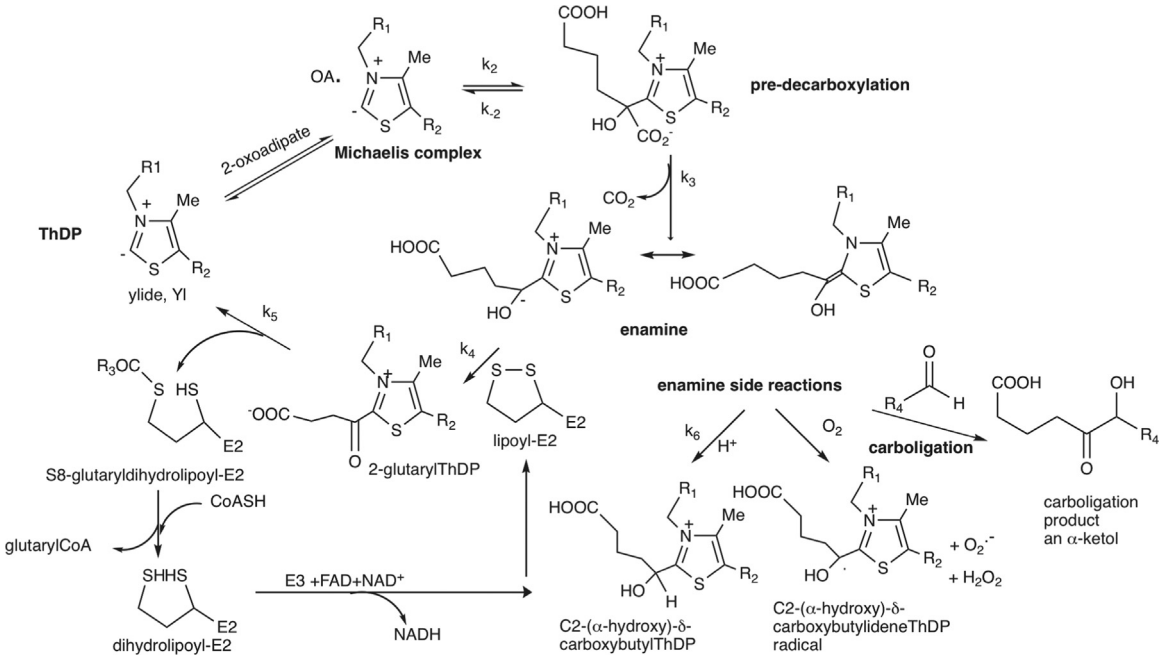

Scheme 1. Mechanism of OGDHc with 2-oxoadipate showing enamine side reactions.

dehydrogenase complex (OADHc) (see Ref. [19] for review). All 2-oxo acid dehydrogenase complexes share the same flavin-containing E3 component in a particular cell, the component, which is suggested to be the major source of superoxide/H_2O_2 generation by mammalian mitochondria [13,14,22,23]. The E3-bound reduced flavin is believed to undergo two single-electron oxidation steps by oxygen, leading to production of superoxide and H_2O_2 [16,24,25]. There are two possible ways to flavin reduction within OGDHc: (i) from OG oxidation in the forward physiological reaction, or (ii) from NADH oxidation in the reverse reaction. Independently of E3, which is not specific for any of the 2-oxo acid dehydrogenase complexes, the hE1o by itself and the hE1o-hE2o sub-complex were also shown to be able to produce superoxide/H_2O_2 from decarboxylation of OG in the physiological direction, with no such activity detected for hE2o by itself [17,18]. The major source of superoxide/H_2O_2 on hE1o was shown to be the ThDP-derived enamine radical, detected by Electron Paramagnetic Resonance Spectroscopy (EPR) in the active centers of both hE1o [17] and *E. coli* E1o [26] upon reaction of E1o's with OG [17]. The specific activity of superoxide and H_2O_2 production by hE1o on its own was less then 1% of the E1o-specific activity [17], and was consistent with the fraction of the radical intermediate species occupying the hE1o active centers (0.2% occupancy for hE1o on its own and 0.59% occupancy for hE1o assembled into hOGDHc). The data indicated that hE1o does produce both the ThDP-enamine radical and superoxide/H_2O_2 that could contribute to oxidative stress involved in Alzheimer's disease pathogenesis, especially under conditions of hE2o deficiency [27,28].

It was of importance to further investigate whether or not the substrate specificity of the hE1o and hOGDHc would tolerate the homologue 2-oxoadipate (OA), differing only by an additional CH_2 group from OG. Recent studies have reported that rat skeletal muscle mitochondria could produce superoxide/H_2O_2 when using 2-oxoadipate as a substrate [29]. This function was assigned to a putative 2-oxoadipate dehydrogenase complex (OADHc), specifically to its FAD-containing E3 component, rather than to OGDHc, due to its potential inhibition at high concentrations of OA [29]. Taking into account that there are already eleven distinct sites known to be associated with superoxide/H_2O_2 generation by mammalian mitochondria [19], it is a great challenge to evaluate each site's individual contribution from OA. Our first question is whether hOGDHc decarboxylates OA with production of NADH and glutaryl-CoA. Earlier, the 2-oxoadipate dehydrogenase activities by OGDH complexes from bacterial [31] and mammalian sources [32,33] were reported but no such studies were reported for the human OGDHc. This is important in view of the controversy regarding which enzyme system, hOGDHc or the putative OADHc is involved in the conversion of OA to glutaryl-CoA in mammalian mitochondria [30,34–36]. Our study is also important to our understanding of the role of hOGDHc in glutaric aciduria type I, the neurodegenerative disorder associated with accumulation of neurotoxic glutaric and 3-hydroxyglutaric acid, that is caused by the deficiency of glutaryl-CoA dehydrogenase, which plays a role in the degradation pathways of L-lysine and L-tryptophan [30,35].

We here report that according to numerous independent experiments, OA behaves very similarly to OG as an alternate substrate (see Scheme 1 for OA), but not inhibitor of the hOGDHc. Perhaps the most significant finding is that H_2O_2 generation efficiency of hE1o and an assembled hOGDHc from OA was about 7-times higher compared to that with the physiological substrate OG under similar conditions. Once more, as with OG, the superoxide/H_2O_2 is being derived from one-electron oxidation of the ThDP-bound enamine intermediate derived from OA on hE1o by O_2. These results also make a significant contribution to our understanding of an important current topic of interest, the efficiency of superoxide/H_2O_2 production from OA by the hOGDHc. Our studies were made possible by our successful expression of the hE1o and hE2o components [17].

2. Materials and methods

2.1. Reagents

ThDP, NAD^+, CoA, DTT and IPTG were from Affymetrix; hydrogen peroxide, peroxidase from horseradish, superoxide dismutase, thiamin·HCl, DCPIP, benzamidine·HCl, 2-oxoglutaric acid and 2-oxoadipic acid were from Sigma-Aldrich; Amplex™ UltraRed was from Invitrogen via Thermo Fisher Scientific.

2.2. Protein expression and purification

Expression and purification of hE1o, hE2o and hE3 were as reported earlier [17].