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The mitochondrial 2-oxoadipate and 2-oxoglutarate dehydrogenase complexes share their E2 and E3 components for their function and both generate reactive oxygen species

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ABSTRACT

Herein are reported unique properties of the novel human thiamin diphosphate (ThDP)-dependent enzyme 2-oxoadipate dehydrogenase (hE1a), known as dehydrogenase E1 and transketolase domain-containing protein 1 that is encoded by the *DHTKD1* gene. It is involved in the oxidative decarboxylation of 2-oxoadipate (OA) to glutaryl-CoA on the final degradative pathway of L-lysine and is critical for mitochondrial metabolism. Functionally active recombinant hE1a has been produced according to both kinetic and spectroscopic criteria in our toolbox leading to the following conclusions: (*i*) The hE1a has recruited the dihydrolipoyl succinyltransferase (hE2o) and the dihydrolipoyl dehydrogenase (hE3) components of the tricarboxylic acid cycle 2-oxoglutarate dehydrogenase complex (OGDHc) for its activity. (*ii*) 2-Oxoglutarate (OG) and 2-oxoadipate (OA) could be oxidized by hE1a, however, hE1a displays an approximately 49-fold preference in catalytic efficiency for OA over OG, indicating that hE1a is specific to the 2-oxoadipate dehydrogenase complex. (*iii*) The hE1a forms the ThDP-enamine radical from OA according to electron paramagnetic resonance detection in the oxidative half reaction, and could produce superoxide and H_2O_2 from decarboxylation of OA in the forward physiological direction, as also seen with the 2-oxoglutarate dehydrogenase hE1o component. (*iv*) Once assembled to complex with the same hE2o and hE3 components, the hE1o and hE1a display strikingly different regulation: both succinyl-CoA and glutaryl-CoA significantly reduced the hE1o activity, but not the activity of hE1a.

1. Introduction

The human thiamin diphosphate (ThDP)-dependent 2-oxoadipate dehydrogenase (hE1a), also known as dehydrogenase E1 and transketolase domain-containing protein 1 (DHTKD1), is believed to be involved in the oxidative decarboxylation of 2-oxoadipate (OA) to glutaryl-CoA, a product on the final degradative pathway of L-lysine, L-hydroxylysine and L-tryptophan [1–7]. The glutaryl-CoA so formed serves as a substrate for glutaryl-CoA dehydrogenase (GCDH: EC 1.3.99.7), which is located downstream of hE1a on the L-lysine degradative pathway and produces glutaconyl-CoA, the precursor for acetyl-CoA [8]. Glutaryl-CoA also serves as a precursor for neurotoxic metabolites such as glutaric acid and 3-hydroxyglutaric acid, which accumulate in glutaric aciduria type I, a metabolic disorder related to GCDH deficiency [9–11]. Recently, mutations were identified in the *DHTKD1* gene that cause 2-aminoadipic and 2-oxoadipic aciduria, inborn disorders which have been associated with various neurological symptoms [2,3,5,6]. The suppression of *DHTKD1* in mitochondria led to a decreased level of ATP and reduced 2-oxoglutarate dehydrogenase complex (OGDHc) activity, as well as to an increased production of reactive oxygen species, suggesting that the *DHTKD1* encoded protein is essential for mitochondrial metabolism [4]. The pharmacological inhibition of the *DHTKD1* was suggested as a promising strategy to treat glutaric aciduria type I [10]. However, the suppression of *DHTKD1* in

Abbreviations: hE1o, 2-oxoglutarate dehydrogenase; hE2o, dihydrolipoyl succinyltransferase; hE3, dihydrolipoyl dehydrogenase; hOGDHc, 2-oxoglutarate dehydrogenase complex; hLDo, lipoyl domain of hE2o; CDo, catalytic domain of hE2o; PSBD, peripheral subunit-binding domain of hE2o; hE1a, 2-oxoadipate dehydrogenase; *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; ROS, reactive oxygen species; OG, 2-oxoglutarate; OA, 2-oxoadipate; FT-MS, Fourier transform mass spectrometry; CD, circular dichroism; EPR, Electron Paramagnetic Resonance

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Dhtkd1-/Gcdh- double knockout mice did not rescue the clinical and biochemical phenotype of glutaric aciduria I, suggesting that an alternative route via the OGDHc may at least partially substitute for the loss of *DHTKD1* function [10]. This hypothesis is in agreement with findings reported by our group recently, which suggested that hOGDHc behaves as a 2-oxoadipate dehydrogenase, in addition to its 2-oxoglutarate dehydrogenase activity in the tricarboxylic acid cycle (TCA) [12]. At present, no studies on the hE1a are available from any source in the literature. Nor is there any information available about the presence of a specific dihydrolipoamide glutaryltransferase component that would suggest that the hE1a is the first component of a novel 2-oxoadipate dehydrogenase complex [13], or evidence for OGDHc-like hybrid complex formation with function via the TCA.

In view of the above information, it was of importance to express recombinant hE1a and to characterize its function using the numerous independent experiments developed in our toolbox for the study of the superfamily of 2-oxo acid dehydrogenase complexes [12,14]. These studies were made possible by our successful expression of the hE1a component, and of the individual components of hOGDHc: the ThDP-dependent 2-oxoglutarate dehydrogenase (hE1o; EC1.2.4.2), dihydrolipoyl transsuccinylase (hE2o; EC 2.3.1.61) and dihydrolipoyl dehydrogenase (hE3; EC1.8.1.4; the E3 component is common to all such complexes in a particular cell) (Scheme 1) [15–18]. The important conclusion from current studies is that the hE1a and the hE1o components could each assemble with the same hE2o and E3 components into the corresponding complexes; they are not functionally redundant, and they both could contribute to the pathologies associated with neurodegenerative disorders, especially those related to oxidative stress in the mitochondria.

2. Material and methods

2.1. Reagents

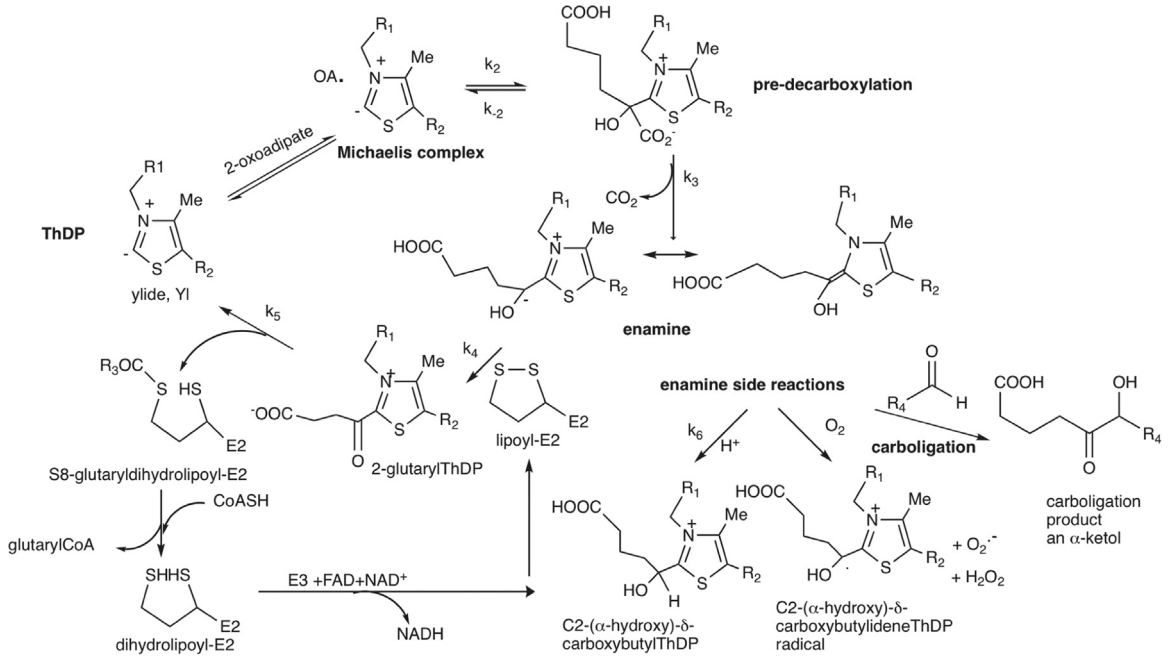
ThDP, NAD^+, CoA, DTT, D,L-α lipoic acid, IPTG, thiamin·HCl, imidazole, DNase I and Micrococcal Nuclease were from Affymetrix; catalase from bovine liver, hydrogen peroxide, peroxidase from horseradish, superoxide dismutase, 2-oxoglutaric acid, 2-oxoadipic acid, 3-methyl-2-oxovaleric acid sodium salt, benzamidine·HCl, glutaryl-CoA lithium salt and succinyl-CoA were from Sigma-Aldrich; Amplex™ UltraRed was from Invitrogen via Thermo Fisher Scientific; Ni Sepharose High Performance was from GE Healthcare.

2.2. Protein expression and purification

Expression and purification of hE1o, hE2o, hE2o catalytic domain (CDo), and hE3 was as reported earlier [19]. Expression and purification of E2o lipoyl domain (LDo) and its lipoylation in vitro by *E. coli* lipoyl protein ligase, was as reported earlier [19].

2.3. Construction of plasmid and expression and purification of hE1a

The gene encoding C-terminally His_6-tagged hE1a was synthesized by ATUM (www.atum.bio Newark, CA). The *DHTKD1* codon optimized for expression in *E. coli* cells was inserted into pET-22b (+) vector through the *Nde*I and *Xho*I restriction sites, and the resulting plasmid was expressed in BL21(DE3) cells. Cells were grown in LB medium supplemented with 50 μg/ml ampicillin containing 1 mM $MgCl_2$ and 0.50 mM thiamin·HCl. Protein expression was induced by 0.5 mM IPTG for 15 h at 18 °C. The harvested cells were dissolved in 50–70 ml of 50 mM KH_2PO_4 (pH 7.5) containing 0.3 M KCl, 2.0 mM $MgCl_2$, 0.25 mM ThDP, 1.0 mM benzamidine·HCl and 0.5% Triton X-100. Cells were treated with lysozyme (0.60 mg/ml) at 4 °C for 20 min. Next, 1000 units of each DNase I, and Micrococcal Nuclease were added and cells were incubated for an additional 20 min at 4 °C. Cells were disrupted using a sonic dismembrator and the clarified lysate was loaded onto a Ni-Sepharose High Performance column (GE Healthcare) equilibrated with buffer A containing 50 mM KH_2PO_4 (pH 7.5), 0.3 M KCl, 2.0 mM $MgCl_2$ and 0.25 mM ThDP. The protein was eluted with buffer A containing 300 mM imidazole and was concentrated. The buffer was exchanged to 0.1 M Tris·HCl (pH 7.5) containing 0.3 M NH_4Cl, 2.0 mM $MgCl_2$, 0.50 mM ThDP and 1.0 mM benzamidine·HCl using a PD-10 desalting column. Protein was frozen in small aliquots in liquid N_2 and was stored at −80 °C. The hE1a amino acid sequence was confirmed by its digestion with pepsin followed by FT-MS detection of the resulting peptides. The peptic digest of hE1a resulted in 85.4% sequence coverage with 80 peptides identified (Table S1).



Scheme 1. Mechanism of hE1a assembled with hE2o and hE3 to complex.