BBA - Molecular Basis of Disease 1864 (2018) 3629–3638

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Molecular and cellular basis of ornithine δ-aminotransferase deficiency caused by the V332M mutation associated with gyrate atrophy of the choroid and retina

Riccardo Montioli[a,1], Maria Andrea Desbats[b,1], Silvia Grottelli[c], Mara Doimo[b,2], Ilaria Bellezza[c], Carla Borri Voltattorni[a], Leonardo Salviati[b,*], Barbara Cellini[c,**]

[a] Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biological Chemistry, University of Verona, Verona, Italy
[b] Clinical Genetics Unit, Department of Woman and Child Health, University of Padova, Via Giustiniani 3, 35128 Padova, Italy
[c] Department of Experimental Medicine, University of Perugia, Perugia, Italy

ARTICLE INFO

Keywords:
Rare disease
Pyridoxal phosphate
Ornithine aminotransferase
Pathogenic mutation
Pyridoxine

ABSTRACT

Gyrate atrophy (GA) is a rare recessive disorder characterized by progressive blindness, chorioretinal degeneration and systemic hyperornithinemia. GA is caused by point mutations in the gene encoding ornithine δ-aminotransferase (OAT), a tetrameric pyridoxal 5′-phosphate-dependent enzyme catalysing the transamination of L-ornithine and α-ketoglutarate to glutamic–γ-semialdehyde and L-glutamate in mitochondria. More than 50 OAT variants have been identified, but their molecular and cellular properties are mostly unknown. A subset of patients is responsive to pyridoxine administration, although the mechanisms underlying responsiveness have not been clarified. Herein, we studied the effects of the V332M mutation identified in pyridoxine-responsive patients. The Val332-to-Met substitution does not significantly affect the spectroscopic and kinetic properties of OAT, but during catalysis it makes the protein prone to convert into the apo-form, which undergoes unfolding and aggregation under physiological conditions. By using the CRISPR/Cas9 technology we generated a new cellular model of GA based on HEK293 cells knock-out for the *OAT* gene (HEK-OAT_KO). When overexpressed in HEK-OAT_KO cells, the V332M variant is present in an inactive apodimeric form, but partly shifts to the catalytically-competent holotetrameric form in the presence of exogenous PLP, thus explaining the responsiveness of these patients to pyridoxine administration. Overall, our data represent the first integrated molecular and cellular analysis of the effects of a pathogenic mutation in OAT. In addition, we validated a novel cellular model for the disease that could prove instrumental to define the molecular defect of other GA-causing variants, as well as their responsiveness to pyridoxine and other putative drugs.

1. Introduction

Human ornithine δ-aminotransferase (hOAT) (E.C. 2.6.1.13) is a pyridoxal 5′-phosphate (PLP)-dependent enzyme that catalyses the conversion of L-ornithine (L-Orn) and α-ketoglutarate (α-KG) to glutamic-γ-semialdehyde (GSA) and L-glutamate, respectively, in the mitochondrial matrix [1]. The spontaneous cyclization of GSA to pirroline-5-carboxylate (P5C) makes the reaction irreversible and supports the main physiological role of the enzyme in L-Orn degradation and in proline synthesis [2,3]. Although the enzyme is ubiquitous, the highest expression levels are found in liver, kidneys, intestine and retina [3].

hOAT is encoded by the *OAT* gene, localized on chromosome 10p26, as a precursor protein of 439 amino acids, endowed with a 25–35

Abbreviations: OAT, ornithine δ-aminotransferase; PLP, pyridoxal 5′-phosphate; GA, gyrate atrophy; L-Orn, L-ornithine; GSA, glutamic-γ-semialdehyde; α-KG, α-ketoglutarate; P5C, pirroline-5-carboxylate; OAT^{wt}, OAT wild-type; OAT^{V332M}, OAT bearing the V332M mutation; PBS, phosphate-buffered saline; ANS, 1-anilinonaphthalene sulphonic acid; PMP, pyridoxamine 5′-phosphate; SEC, size-exclusion chromatography; $K_{D(dim\text{-}tet)}$, dimer-tetramer equilibrium dissociation constant; CD, circular dichroism; HEK, human embryonic kidney; HEK-OAT_KO, HEK293 cells knock-out for the *OAT* gene

* Correspondence to: L. Salviati, Clinical Genetics Unit, Department of Woman and Child Health, University of Padova, Padova, Italy.
** Correspondence to: B. Cellini, Department of Experimental Medicine, University of Perugia, P.le Gambuli 1, 06132 Perugia, Italy.
E-mail addresses: leonardo.salviati@unipd.it (L. Salviati), barbara.cellini@unipg.it (B. Cellini).
[1] The authors contributed equally to this work.
[2] Current address: Department of Medical Biochemistry and Biophysics, Umeå University, SE-901 87 Umeå, Sweden.

https://doi.org/10.1016/j.bbadis.2018.08.032
Received 26 May 2018; Received in revised form 27 July 2018; Accepted 24 August 2018
Available online 26 August 2018
0925-4439/

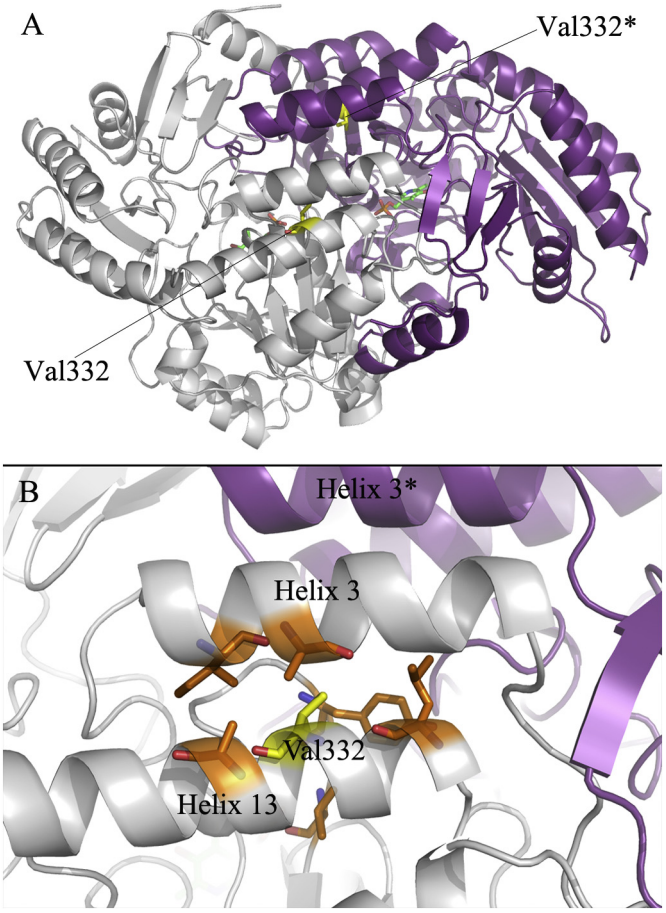

Fig. 1. Structure of the hOAT dimer. Ribbons representation of the hOAT dimer. The two monomers are colored purple and white, respectively. (A) Global view of the hOAT dimer in which Val 332 residues are colored yellow and PLP molecules are colored green. (B) Detail of helices 3 and 13. The position of Val332 is indicated and residues belonging to the hydrophic cleft are highlighted as orange sticks. *Indicate residues belonging to the neighboring subunit. The image was rendered using the PyMol software (Schrödinger).

residues mitochondrial targeting sequence, which is cleaved upon import giving the final mature form of the protein [4–7]. The crystal structure of the enzyme has been solved both in the free form and in complex with known inhibitors [8–11]. It belongs to the Fold Type I class of PLP-dependent enzymes (Fig. 1A). In each monomer of the dimeric unit an N-terminal segment forms a α-helix, which partly overlaps with the large domain of the neighbouring subunit, and a three-stranded β-sheet. The large domain (residues 95–344) comprises the active site region and most of the subunit interface, while residues 345–439 generate a small C-terminal domain [9]. The active site is located in a cleft close to the monomer-monomer interface. PLP is held in the active site by residues belonging to both subunits and forms a Schiff base with the ε-amino group of Lys292. This complex, called internal aldimine, gives rise to typical absorbance and dichroic bands at 420 and 340 nm, corresponding to the ketoenamine and the enolimine tautomer, respectively [9].

Although the crystallographic conditions favour a hexameric assembly, studies performed in solution have confirmed that hOAT displays a tetrameric quaternary structure. Indeed, the functional unit of the enzyme is the dimer, as demonstrated by the kinetic properties of the dimeric R217A variant [12]. Apo- and holoOAT show a similar tertiary structure, but in the holo-form the dimer-tetramer equilibrium is shifted toward the tetramer. Moreover, the apo-form displays a significantly decreased thermal stability, and is prone to unfolding and aggregation under physiological conditions. These data have led to the hypothesis that the coenzyme induces a conformational change in OAT that increases the resistance of the enzyme against thermal denaturation and possibly promotes its stability in the cellular environment [12].

Mutations of the *OAT* gene (10q26) cause the rare recessive disorder gyrate atrophy (GA), a disease characterized by the progressive degeneration of the choroid and retina beginning in childhood and leading to blindness on the fourth to fifth decade of life. GA patients also exhibit hyperornithinemia and occasionally display signs of mental retardation [13]. The molecular mechanisms of the disease are still unclear, but it has been suggested that retinal degeneration could depend on both the accumulation of toxic ornithine derivatives and the deficiency of proline [14,15]. GA is present worldwide, with a particularly high incidence in Finland (1:50000) [16]. The disease is currently treated with an arginine-restricted diet, which aims at reducing the production of ornithine, or with the supplementation with Vitamin B6, which increases the plasmatic levels of PLP and is able to reduce ornithinemia in a minority of the patients [17,18].

About 50 disease-causing mutations have been identified until now, including non-sense, frameshift and missense mutations, the latter being the most frequent [19]. Missense mutations involve residues spread over the OAT structure and in most cases located far from the enzyme active site [9,20], thus suggesting that various defects could lead to the enzymatic deficit. Studies performed in yeast have revealed that some mutations reduce OAT specific activity, while others interfere with the proper oligomerization of the protein and/or with its intracellular stability [21]. However, the molecular and cellular behaviour of most GA-causing variants is currently unknown, and the unavailability of suitable cell models of disease has certainly limited major advances in the field.

The substitution of Val332 with a methionine residue is one of the first identified in B6-responsive GA patients [18]. The ornithine transaminase activity of the V332M variant (OAT^{V332M}) expressed in mammalian cells was found to be negligible in the absence of added coenzyme, but became clearly visible upon addition of excess PLP [18]. In yeast, the variant is expressed at levels similar to OAT wild-type (OAT^{wt}), although the enzymatic activity is not detectable, and it shows an altered electrophoretic pattern under native conditions indicative of possible influences of the mutation on the quaternary structure [21]. Similar results were also observed in patient fibroblasts. Val332 is located far from the active site (Fig. 1A and B) and its substitution with a methionine residue could perturb the folding of human OAT, but a detailed characterization of OAT^{V332M} is lacking.

In the present work we confirmed that OAT^{V332M} in the recombinant purified form is not endowed with a catalytic defect, but during catalysis converts from the holo- to the apo-form, which is prone to unfolding and aggregation. By applying the CRISPR/Cas9 genome editing technology we generated a cellular model of GA based on human embryonic kidney (HEK) 293 cells knock-out for the *OAT* gene. OAT^{V332M} expressed in HEK293 knock-out cells is mainly present in an inactive apodimeric form. The PLP coenzyme prevents the aggregation of purified $apoOAT^{V332M}$ and seems to increase its specific activity at a cellular level. The significance of the data obtained for a better understanding of both GA pathogenesis and the response of the patients to B6 administration is discussed.

2. Materials and methods

2.1. Materials

PLP, L-Orn, α-KG, 2-aminobenzaldehyde, dimethyl sulphoxide (DMSO), isopropyl-β-D-thiogalactoside (IPTG) and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma-Aldrich. 1,8-Anilino-naphthalene sulfonic acid (ANS) was purchased from Molecular Probes. All other chemicals were of the highest purity available.