Structure analysis of the disease-causing variants of the human dihydrolipoamide dehydrogenase

Ph.D. thesis booklet

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INTRODUCTION

(Dihydro)lipoamide dehydrogenase (LADH, E3, L protein, EC 1.8.1.4) is a member of the pyridine nucleotide-disulfide oxidoreductase enzyme family. In physiological conditions, the LADH catalyzes the oxidation of dihydrolipoamide (DHLA) using NAD^+ as a co-substrate in a reversible reaction.

LADH is a common subunit of the mitochondrial alpha-keto acid dehydrogenase multienzyme complexes and the glycine cleavage system (GCS). LADH plays crucial roles in metabolism by catalyzing the oxidation of the covalently linked DHLA cofactor of the above complexes, and re-establishing initial conditions in the complexes after each catalytic turnover. As part of the pyruvate dehydrogenase (PDHc) and alpha-ketoglutarate dehydrogenase (KGDHc) complexes, LADH is a key enzyme in the aerobic carbohydrate metabolism and the citric acid cycle, respectively. As part of the alpha-ketoadipate dehydrogenase complex (KADHc), the branched chain alpha-keto acid dehydrogenase complex (BCKDHc) and the GCS, LADH is also involved in the metabolism of several amino acids. Of note, the regulation of the above complexes by the $NADH/NAD^+$ ratio – in connection with the metabolic status of the cells – proceeds *via* regulation of the LADH component.

The LADH-reaction requires an FAD prosthetic group, a redox-active disulfide and the catalytic base in the active site. The LADH is a functional or obligate homodimer. According to the crystal structures, one monomer provides the Cys45-Cys50 redox pair (according to the amino acid numbering of the human enzyme), the FAD prosthetic group and the amino acids for its stabilization, as well as the NAD^+/NADH binging site. The adjacent monomer carries the His452′ (′ stands for amino acids that belong to the adjacent monomer) functioning as the catalytic base, and the amino acids that

establish the proper orientation and pKa of the latter. The dihydrolipoamide/lipoamide-binding site (from now on referred to as the LA-binding site) and its more hydrophilic continuation that probably serves as an outlet for H^+/H_3O^+ and/or H_2O upon the enzymatic reaction (hence referred to as the H^+/H_2O channel) are both located on the dimer interface.

The LADH exhibits ping-pong kinetics and the reductive and oxidative half-reactions take place on the two opposite sides of the isoalloxazine ring system of the FAD, hence they are spatially separated. In the forward direction of the physiological reaction DHLA substrate reduces the Cys45-Cys50 disulfide of the oxidized LADH. In the two-electron reduced state (EH_2) of LADH electrons are shared among the two cystein thiols and the FAD; predominantly a charge-transfer complex between the Cys50 thiolate anion and the FAD is formed. In the second half-reaction, a hydride equivalent is transfered to NAD^+ *via* the FAD prosthetic group and H^+ is released. H452′ is responsible to deprotonate the DHLA substrate and stabilize the charge transfer complex. In the reverse direction of the LADH-reaction, NADH reduces the enzyme and the transfer of electrons is reversed: first FAD is reduced, and the thiolate-FAD charge transfer complex and the dithiol states are formed only subsequentially. The catalytic cycle can be interrupted, and hence the LADH-activity inhibited by sulfhydryl reagents that hinder the formation of the charge transfer complex, or by the four-electron reduction of LADH by NADH.

In addition to its physiological importance, LADH also possesses diaphorase and oxidase activities using alternative electron acceptors. Conditions that facilitate the formation of the fully reduced state of FAD in LADH (such as four-electron reduction by NADH or in the presence of sulfhydryl agents) not only inhibit the physiological activity, but also also enhance the diaphorase and oxidase activities.

The oxidase reaction results in the formation of reactive oxygen species (ROS) – superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxyde (H_2O_2) – and therefore is of pathological significance. The human (h) LADH can generate ROS not only as a free enzyme, but also as part of multienzyme complexes, primarily the hKGDHc. hKGDHc proved to be a major contributor to mitochondrial oxidative stress under pathologically relevant conditions, such as low pH and elevated $NADH/NAD^+$ ratio. ROS generation of hKGDHc is primarily attributed to the LADH subunit.

Pathogenic mutations to the hLADH-coding *DLD* gene result in inactive or partially inactive protein variants that affect several central metabolic pathways simultaneously and lead to the often prematurely lethal genetic disease called hE3 deficiency. The 14 disease-causing substitutions/deletion reported to date may affect different functional regions of the enzyme. However, localization did not show correlation with loss in LADH-activity. The phenotypes of E3-deficiency are versatile and range from early-onset neurologic manifestations to adult-onset isolated liver involvement. The recurrent metabolic episodes are frequently associated with hypoglycemia, lactate acidosis, hyperammonemia, and hepatomegaly; liver failure can result in death, even in those with late-onset disease.

LADH-activites were generally decreased when measured either in tissue samples from patients with E3-deficincy or using isolated recombinant hLADH variants *in vitro*. However, the severity of the disease generally did not correlate well with the loss in LADH function measured from patients. Hence other auxiliary biochemical mechanisms must also contribute to the pathogenesis. Recent results suggest that such mechanisms involve the enhanced ROS generation by selected disease-causing hLADH variants (especially in acidosis), the dissociation of selected variants from the harboring multienzyme complexes contributing to their impaired function, and perhaps also

the ROS production of the E1-E2 subcomplex of hKGDHc upon dissociation of a pathogenic hLADH variant from hKGDHc.

The only experimental structure analysis of the pathogenic hLADH variants thus far was a hydrogen-deuterium-exchange mass spectrometry (HDX-MS) study performed on ten mutants. In order to better understand the structural basis for the altered enzymatic activites and the molecular pathomechanisms of E3-deficiency associated with the disease-casuing substitutions, high-resolution structural data are needed.

OBJECTIVES

The primary goal of my research was to determine the structures of the hLADH and its disease-causing variants using X-ray crystallography. With the crystal structures in hand, I aimed to reveal the molecular pathomechanisms that underlie E3-deficiency. Substitutions residing in different functional regions of hLADH were investigated. Specific and ROS-generating activities of those pathogenic variants that have not been studied in our laboratory before were also to be determined, so that structure-function correlation could be established based on functional studies carried out among identical conditions.

Additionally, specifically designed hLADH variants were to be studied functionally, in order to clarify the potential roles in catalysis of the Glu332 and Arg460 amino acids, and thus to confirm the crystallographic results and potentially reveal unknown features of the LADH-reaction.

METHODS

Construction of plasmid vectors

Heterologus protein expression was done using pET52b(+) plasmid vectors. The cDNA of the LADH-coding *DLD* gene was codon-optimized for *E. coli* expression and cloned into the plasmid without the mitochondrial signal sequence. The plasmid vector provided a Strep affinity tag fused to the N-terminal end of the protein, and the gene of β-lactamase as a selection marker.

Single point mutations were introduced by the QuikChange II XL Site Directed Mutagenesis Kit according to the manufacturer's specifications using the wild type plasmid. Primers were designed by the QuikChange Primer Design program and purchased as HPLC purified oligonucleotides. Mutagenesis products, *i.e.* plasmids with the mutated hLADH sequence, were transformed into One Shot TOP10 competent *E. coli* cells and isolated using the QIAprep Spin Miniprep Kit. Successful mutagenesis was confirmed by DNA sequencing.

Recombinant protein expression and purification

hLADH and its variants were expressed using pET52b(+)/BL21(*DE3*) system and purified by Strep-tag based affinity chromatography. The protocol applied for the protein isolation was developed earlier in our laboratory. Purity of the protein products was verified by SDS-PAGE, whereas amino acid sequences were all verified by MS analysis. Proteins were flash-frozen in liquid nitrogen and kept at −80°C until use.

Protein crystallography

Crystallization trials were carried out applying the sitting drop vapor diffusion method and using preformulated screening solutions purchased from Quigen and Hampton Research. Crystal Screen #39 (Hampton Research, 2 M (NH4)2SO4, 2 v/v% PEG 400, 0.1 M Hepes, pH 7.5) provided three-dimensional, well-diffracting crystals for R447G-, I445M-, and G426E-hLADH. Optimization of the crystallization conditions were needed for all the other proteins.

hLADH could be successfully crystallized by the optimization of the Index Screen #83-85 solutions (Hampton Research); data set used for structure determination derived from crystal grown under 0,2 M $MgCl_2$, 25 m/v% PEG 3350 and 0,1 M Bis-Tris (pH 7.45).

Crystallization of G194C-, R460G-, and P453L-hLADH was achieved by the optimization of the above mentioned Crystal Screen #39 condition. The compositions of the final crystallization solutions that resulted in the best diffracting crystals for these variants were: 2 M $(NH_4)_2SO_4$, 2 v/v% PEG 400, 0.1 M Bis-Tris (pH 6.9) for G194C-hLADH; 2 M $(NH_4)_2SO_4$, 2 v/v% PEG 400, 0.1 M Bis-Tris (pH 6.5) for R460G-hLADH; 2 M $(NH_4)_2SO_4$, 1.5 v/v% PEG 400, 0.1 M Hepes (pH 7.3) for P453L-hLADH.

On the other hand, optimization of the Na/K-phosphate containing solutions of the Qiagen NeXtal pHClear II led to crystallization of the D444V-hLADH; final condition consisted solely of 16 M Na/K-PO_4 (pH 8.1).

Crystals were flash-frozen and stored in liquid nitrogen until data collection; cryo protection was used for hLADH (Parabar 10312) and for D444V-hLADH (1.8 M Na/K-PO_4 (pH 7.5), 20 v/v% glycerol).

Structure determination and analysis

Diffraction data were collected using synchrotron radiation on the BL14.1 beamline at the BESSY II electron storage ring operated by Helmholtz-Zentrum Berlin. Diffraction data were processed on site using XDSAPP 2.0 in parallel with the data collection.

Phase problem was solved by molecular replacement in Molrep using chain A of a previously determined hLADH structure (PDB ID: 1ZMD) as a search model. For all structures, rigid body refinement was carried out using Refmac5 of the CCP4 sotware suite, followed by repeated cycles of restrained refinement and real space model (re-)building in Coot. Restrained refinement cycles were all carried out in Refmac5 for D444V-hLADH, while first Refmac5 and then phenix.refine in Phenix were used for all other model structures. Model structures were validated by Molprobity connected to phenix.refine. The coordinates and corresponding structure factor amplitudes of the structures of the wild type hLADH, as well as the D444V-, P453L-, G194C-, R460G-, R447G-, I445M-, and G426E-hLADH variants have been deposited in the RCSB Protein Data Bank, with accession numbers 5NHG, 5J5Z, 6I4Z, 6I4P, 6I4R, 6I4S, 6I4T, and 6I4U, respectively.

Pathogenic variants were compared with one of the two hLADH structures determined by our group (PDB ID: 6I4Q) (in the determination of which I took part, but I do not present here as my own result; see also Results for more details). Least-squares fitting of the variant structures (whole dimer) onto the wild type structure (whole dimer) and calculations of the overall and residue-level root-mean-square deviation (RMSD) values were performed using ProFit. Dimer interfaces and protein-FAD interactions were characterized using the online PISA (Proteins, Interfaces, Structures, Assemblies) server and the CONTACT program of CCP4. Geometry of the LA-

binding and H^+/H_2O channels were examined by the Caver Analyst 2.0 program; the starting point for tunnel calculation was always the center of gravity between the Cys45(SG; in disulfide bond) and His452′(NE2) atoms in the active site, except occasionally for P453L-hLADH, where the two atoms were Cys45(SG) and His452′(CE1) in case the His452′ imidazole ring was flipped. Visualization and analysis of surface polarities and conformational changes of amino acid side chains were performed in PyMol.

Enzyme activity measurements

Protein concentrations were determined by the colorimetric Bradford method, which is not affected by FAD contributions (FAD does not absorb at 595 nm). Seven-point linear reference curves were constructed using bovine serum albumin solutions with concentration ranging from 0.1 to 1.4 mg/ml.

Specific activities of hLADH and its variants were assessed spectrophotometrically in both the forward and reverse directions by measuring the production or consumption, respectively, of NADH at 340 nm. For each experiment, a 300 µl final reaction volume in 50 mM K-PO_4, pH 7.3 was used containing 165 µM NAD^+, 0.9 mM dihyrolipoic acid and 123 ng protein (forward direction), or 165 µM NADH, 0.9 mM lipoamide and 12.3 ng protein (reverse direction). Reactions were initiated by the addition of dihydrolipoic acid (forward) or NADH (reverse) after 15 min incubation at 37°C.

Superoxide generation by hLADH and its variants was measured spectrophotometrically by reduction of partially acetylated cytochrome c at 550 nm. Solution conditions were the following in a 200 µl final reaction volume: 50 mM K-PO_4, pH 6.3, 50 µM acetylated cyt c, 165 µM NADH, 2.47 µg protein. Reactions were initiated by the addition of NADH after 15 min incubation at 37°C.

Statistical differences in enzyme activites were evaluated with two-tailed Student's t-tests assuming unequal variances and were accepted to be significant when $P < 0.05$.

RESULTS

The structure of the wild type hLADH

Our research group has published two uncomplexed and unliganded structures of the hLADH (PDB IDs: 5NHG and 6I4Q). During my Ph.D. work, I took part in the determination of both two structures, but I only consider the determination of the 5NHG structure as my own result. However, I carried out the detailed analysis of both two structures and these results represent my work.

The two new structures of hLADH have not changed our previous understanding of the overall structure or mechanism of action. In the hLADH structure at 1.75 Å resolution (PDB ID: 6I4Q), two amino acids of the H^+/H_2O channel with charged functional groups in their side chains, namely the Glu332 and the Arg460′, were found to dynamically control the geometry, location of the bottleneck and charge distribution of the channel by adopting two alternative conformations. In the presence of one of the two possible conformers of Arg460′ (Arg460′[A]), Glu332 is forming the bottleneck of the channel (in both two alternative conformations) together with highly conserved amino acid side chains (in [A] or [B] conformations with Glu457′ and Asn473′, or His452′ and Tyr19, respectively) that have been proved to be involved in the catalityc activity and/or substrate binding, as well as in maintaining the structural integrity of the active site. Transition from Arg460′[A] to Arg460′[B] significantly decreases the inner diameter in the vicinity and a new bottleneck is formed, regardless of the Glu332 conformation. Additionally, Arg460′ interconnects two structurally crucial α-helices by forming salt-bridges with Asp333 and Glu332, and therefore greatly contributes to the integrity of the H^+/H_2O channel.

The hLADH structure at 2.27 Å resolution (PDB ID: 5NHG) provided new information regarding the surface region that interacts

with the respective subunit binding domains of the E3-binding protein (hE3BP) in the hPDHc, and the E2 subunit of the hBCKDHc ($hE2_{BCKDHc}$). The 5NHG hLADH structure displayed variability in this region, since the hydroxyl group of Tyr438 interacted with Asp444′ in two ways: Tyr438 H-bonded with the main chain carbonyl group of Asp444′ in the A-B and E-F homodimers, but with the side chain carboxyl group of Asp444′ in the C-D and G-H homodimers (four homodimers were found in the assymetric unit of the 5NHG hLADH structure). The latter Tyr438-Asp444′ interaction is more favourable for the π-π type stacking interaction of Tyr438-Tyr438′ and is stabilized upon binding to hE3BP or $hE2_{BCKDHc}$.

The structures of seven disease-causing hLADH variants

Crystal structures of seven disease-causing hLADH variants were determined in order to analyze the structural basis of E3 deficiency. The investigated substitutions reside in either the active site (P453L), the NAD^+/NADH-binding region (G194C), or the dimer interface (G426E, D444V, I445M, R447G, and R460G). Resolutions of the structures ranged from 1.44 to 2.34 Å. The structures were compared against the wild type hLADH determined at 1.75 Å (PDB ID: 6I4Q) because this structure offered higher resolution, and because it was crystallized under similar conditions resulting in nearly identical crystal packing and therefore good comparability.

Among the seven investigated pathogenic variants, P453L-hLADH proved to be the most structurally different from the wild type. The substitution directly affected Pro453 that forms a *cis* peptide bond with the the adjacent His452 and therefore is essential for the proper orientation of the catalytic base. In P453L-hLADH, the catalytic His452 is in a *trans* peptide bond with Leu453 that led to the displacement and conformational change of His452. As a result,

several crucial interactions were also affected: neither the H-bond between the carbonyl oxygen of His452 and the N3 atom of the isoalloxazine group of the FAD′, nor the His452-Glu457 salt bridge could be formed. The P453L substitution also triggered changes near the FAD isoalloxazine ring. The most striking among these was the significant displacement of Arg393 towards the FAD′ and Lys54′ that is essential in stabilizing FAD′ *via* H-bonding. Additional alterations in the LA-binding and H^+/H_2O channels were also apparent and they could affect the accessibility of the active site.

The structure of the G194C-hLADH variant was remarkably similar to the wild type hLADH. The substitution perturbs a highly conserved helix (Val188-Gly201) whose N-terminus provides residues to the NAD^+/NADH-binding site, more precisely the nicotinamide-binding site. Despite the critical location, the nicotinamide-binding residues suffered no significant structural changes in G194C-hLADH, except a few amino acids showing altered conformation in the immediate vicinity of the substituted residue. The G194C substitution induced no direct alterations to the active site, the LA-binding site, or the FAD-binding residues; in the H^+/H_2O channel, the sole alteration is the lost conformational flexibility of the Arg460 residue.

The G426E substitution takes place in the dimer interface region, in the spatial vicinity of the nicotinamide-binding site. Glu426 is spatially located in between two other glutamates (Glu363 and Glu427) in the pathogenic variant. Therefore, the Glu427-Arg393′ and Glu363-Arg393′ salt bridges present in the wild type cannot be formed due to steric reasons, and a newly formed Glu426-Arg393′ interaction could be observed in the structure of G426E-hLADH. Presence of the Glu426 side chain also introduced significant conformational dynamics locally: the Glu426 and Glu363 possessed alternative conformers that appeared to be “coupled” for steric

reasons. Even though all these structural changes occured in the direct proximity of the nicotinamide-binding site, the G426E substitution induced no significant structural alterations to the cofactor-binding residues relative to wild type hLADH. In G426E-hLADH, the active site, the LA-binding channel, and the FAD-binding residues were unaffected, and also the H^+/H_2O channel appeared to be mostly unaltered.

The D444V, R447G, and R460G substitutions all resulted in the loss of ionic contacts between the two monomers, while the I445M substitutiton affected a hydrophobic region of the interface. Our structural data revealed that the above four pathogenic substitutions reside in the vicinity of the the H^+/H_2O channel and led to perturbations in its geometry and/or polarity to different degrees. The Arg460 and Arg447 residues bear charged functional groups in their bulky side chains and have a key role in maintaining the structural integrity of the H^+/H_2O channel, therefore the R460G and R447G substitutions led to a wider channel segment with increased negative surface potential locally. Conformational flexibilities of the Glu332 and Arg460 residues were affected in the D444V-, I445M-, and R460G-hLADH variants. In the D444V-hLADH and the I445M-hLADH structures only Glu332′[B] and Arg460[A], or Glu332′[A] and Arg460[B] conformations were present, respectively, while in R460G-hLADH only Glu332′[A] could be observed beside the missing arginine side chain. Additionally, C-terminal segment of the channel forming Leu327′-Gly344′ α-helix was slightly displaced in the D444V-, I445M-, and R460G-hLADH variants due to loss of the stabilizing salt-bridges. None of the above discussed four pathogenic substitutions led to displacements of catalytic or FAD/NAD^+/NADH-binding residues.

Enzyme activities of the G426E-, I445M-, and R447G-hLADH variants

To study the structure-function relationships, three variants (G426E-, I445M-, and R447G-hLADH) were also subjected to *in vitro* enzyme activity measurements. The same experimental conditions were used as for the other four mutants investigated earlier in our laboratory (P453L-, G194C-, D444V-, and R460G-hLADH), so that the results are comparable.

The amino acid substitutions generally induced more significant decrease in LADH-activity in the reverse direction of the specific reaction compared to the forward direction. The G426E substitution increased the LADH-activity in the forward direction by 10% (unique among the investigated mutants), but it decreased it in the reverse direction to 36% relative to the wild type. The I445M-hLADH variant also showed a significant difference in the residual activities in the two directions of the LADH-reaction: activity was 77 or 16% relative to the wild type in the forward or the reverse directions, respectively. On the other hand, the R447G substitution lowered the specific activity to a similar degree, 69 or 55% relative to the wild type in the forward or the reverse direction, respectively. None of the three variants exhibited increased ROS-production when compared to wild type.

Enzyme activities of the Glu332- and Arg460-substituted hLADH variants

To investigate the possible roles of the Glu332 and Arg460 residues of hLADH and to understand the pathomechanism of the disease-causing substitutions that affect the H^+/H_2O channel,

additional hLADH variants were generated and their specific and ROS-generating activities were measured.

Substitution of Glu332 with alaine, aspartate or glutamine reduced the LADH-activity in a parallel fashion in the forward and reverse directions, but to different degrees among each other. The E332A substitution lowered the LADH activity by 43 and 39% in the forward and reverse catalytic directions, respectively. The enzymatic activity was even more compromised by E332D in both catalytic directions (59 or 70% decrease in the forward and reverse catalytic directions, respectively), but was better retained by E332Q (31 or 11% decrease in the forward and reverse catalytic directions, respectively). ROS-generating activity was not affected by the E332A substitution, but 39% decrease and 18% increase was observed with the E332D- and E332Q-hLADH variants, respectively.

The R460A, R460E, and R460K substitutions affected the LADH activity differently in the reverse direction (68, 51, and 161%, respectively), but similarly and only modestly in the forward direction (89%, 81%, and 72%, respectively). ROS generations by the above three variants were all lower compared to the wild type.

CONCLUSIONS

Structural alteration caused by amino acid substitutions associated with E3-deficiency

Crystal structures of the hLADH and seven of its disease-causing variants were determined and analysed in order to reveal the structural basis of the impaired enzymatic functions and the E3-deficiency. The following conclusions were drawn:

1. P453L substitution affecting the active site directly and leading to almost complete loss in enzyme activity induced extensive structural alterations to the active site, presumably rendering deprotonation and/or binding of the DHLA substrate impossible. Increased ROS-generating capacity of the P453L-hLADH variant can presumably be attributed to the structural changes around the FAD prosthetic group and the proposed locus of superoxide generation, as well as with diminished interactions that not only stabilize FAD, but also modulate its redox potential.
2. G194C-hLADH displayed the least deviations in structure without inducing alterations to the nearby cofactor binding residues, which is in accord with the retained LADH-activity. Similarly, the G426E substitution altered the charge distribution and dynamics near the nicotinamide-binding site, but did not induce significant structural changes, which may explain the decrease and (slight) increase in LADH-activity in the revese and forward directions, respectively.
3. The D444V, I445M, R447G, and R460G substitutions all reside farther away from the active site on the homodimer interface of hLADH. However, they are still associated with significant losses in catalytic activity (except for the LADH-activity of I445M-hLADH in the forward direction). Our structural data of the four

variants above revealed considerable perturbations in the so-called H^+/H_2O channel leading to the active site, generally with the involvement of Glu332. The significant losses in LADH-activity and the often enhanced ROS-producing activities of the latter variants could only be interpreted by presuming additional effects acting on the active site, the pKa of the catalytic base and/or the redox potential of the FAD prosthetic group, mediated perhaps by local dynamics and/or dipole moment alterations in selected nearby α-helices.

Newly revealed features regarding the structure and catalyzed reaction of hLADH

The Glu332 and Arg460 residues were found to possess two alternative conformations in the wild type hLADH and therefore were suggested to modulate the geometry and polarity of the H^+/H_2O channel. Conformational flexibilities of the Glu332 and Arg460 residues were affected in the D444V-, I445M-, and R460G-hLADH variants. The potential roles in catalysis of the two amino acids mentioned above were investigated further by measuring the specific and ROS-generating activities of specifically designed hLADH variants. Based on these results, the following conclusions were drawn:

4. The role of the side chain carboxyl group of Glu332 could be partially maintained with an amide group. However, its complete absence or improper position resulted in significant decrease in enzyme activity. Glu332 likely contributes to the stabilization of the substrate or a reaction intermediate in the active site and/or H^+/H_3O^+ translocation in the course of the catalytic cycle; altered conformation of the Glu332 residue therefore could be associated

with the molecular pathomechanisms of the D444V-, I445M-, and R460G-hLADH variants.

5. Arg460 presumably plays a rather passive role. The side chain of Arg460, which connects two structurally crucial α-helices, is likely important for maintaining the integrity of the active site and/or modulating the redox potential of the FAD prosthetic group. Based on our results, no active catalytic role could be attributed to the guanidino group of Arg460.

LIST OF PUBLICATIONS

Publications related to the PhD thesis:

Szabo E, Wilk P, Nagy B, Zambo Z, Bui D, Weichsel A, Arjunan P, Torocsik B, Hubert A, Furey W, Montfort WR, Jordan F, Weiss MS, Adam-Vizi V, Ambrus A. (2019) Underlying molecular alterations in human dihydrolipoamide dehydrogenase deficiency revealed by structural analyses of disease-causing enzyme variants. Hum Mol Genet 28(20): 3339-3354.
IF: 5,100

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IF: 5,657

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without IF

Enzyme activity measurements were carried out in the course of a project supported by the New National Excellence Program (ÚNKP grant, 2019/2020, 5 months), and results have not been published yet.

Other publications:

Jako T, **Szabo E,** Tabi T, Zachar G, Csillag A, Szoko E. (2014) Chiral analysis of amino acid neurotransmitters and neuromodulators in mouse brain by CE-LIF. Electrophoresis 35(19): 2870-2876.
IF: 3,028