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Review

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First insights into structure-function relationships of alkylglycerol monooxygenase

Abstract: Alkylglycerol monooxygenase is a tetrahydrobiopterin-dependent enzyme that cleaves the *O*-alkyl-bond of alkylglycerols. It is an exceptionally unstable, hydrophobic membrane protein which has never been purified in active form. Recently, we were able to identify the sequence of alkylglycerol monooxygenase. TMEM195, the gene coding for alkylglycerol monooxygenase, belongs to the fatty acid hydroxylases, a family of integral membrane enzymes which have an 8-histidine motif crucial for catalysis. Mutation of each of these residues resulted in a complete loss of activity. We now extended the mutational analysis to another 25 residues and identified three further residues conserved throughout all members of the fatty acid hydroxylases which are essential for alkylglycerol monooxygenase activity. Furthermore, mutation of a specific glutamate resulted in an 18-fold decreased affinity of the protein to tetrahydrobiopterin, strongly indicating a potential important role in cofactor interaction. A glutamate residue in a comparable amino acid surrounding had already been shown to be responsible for tetrahydrobiopterin binding in the aromatic amino acid hydroxylases. Ab initio modelling of the enzyme yielded a structural model for the central part of alkylglycerol monooxygenase where all essential residues identified by mutational analysis are in close spatial vicinity, thereby defining the potential catalytic site of this enzyme.

Keywords: alkylglycerols; etherlipids; tetrahydrobiopterin.

Enzymes: alkylglycerol monooxygenase (1-alkyl-sn-glycerol; tetrahydrobiopterin:oxygen oxidoreductase [EC 1.14.16.15]).

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Introduction

Phenylalanine degradation, neurotransmitter synthesis, alkylglycerol cleavage and production of the vessel relaxant nitric oxide all share a common feature: the cofactor tetrahydrobiopterin, which is needed for catalysis of these processes (for a review, see [1]). This metabolite is structurally related to the vitamins folate and riboflavin, our body however has still conserved the ability to synthesise this cofactor from guanosinetriphospate in a three-step enzymatic cascade headed by the rate-limiting enzyme GTP cyclohydrolase I. Tetrahydrobiopterin is crucially involved in the activity of eight different enzymes, that is, phenylalanine hydroxylase, tyrosine hydroxylase, two tryptophan hydroxylases, three nitric oxide synthases (neuronal, inducible and endothelial) and alkylglycerol monooxygenase (for a review, see [1]). Whereas the former seven enzymes have been extensively studied over the past decades since their first description [2–7], alkylglycerol monooxygenase characterisation was hampered because of its high hydrophobicity and its extreme lability in standard biochemical assays, even though its first description appeared in the literature long before most of the other enzymatic reactions [8]. The only information available up to 2010 was based on studies in homogenates from animal tissues displaying high activities and gave some insight into the

distribution of the enzyme in the animal body, substrate specificity and the enzymatic reaction catalysed ([9–11]; for a review, see [12]).

Alkylglycerol monooxygenase sequence assignment

When we took over the project to study alkylglycerol monooxygenase in more detail, we were therefore confronted with different challenges. First, a more sensitive assay for the enzyme was needed in order to determine tiny amounts present in cells or tissues. Second, and most crucial, a suitable detergent had to be identified which would help the enzyme to keep its catalytic activity even under biochemical purification protocols which would then allow to enrich the protein over several steps. By this, we were hoping to be able to identify the protein sequence by means of mass spectrometry.

The sensitive assay was developed based on a specifically synthesised fluorescent alkylglycerol, 1-*O*-pyrenedecylglycerol, which was readily accepted by the enzyme and cleaved in the corresponding pyrene labelled aldehyde, which is converted in cellular and tissue homogenates to pyrenedecanoic acid. Substrate and product were separated by HPLC and detected by fluorescence with a detection limit of 4 fmol [13].

The second challenge was more difficult. A whole range of detergents were tested but none proved to be satisfactory for biochemical protocols. Digitonin in concentration of 2% achieved some solubilisation of the enzyme but gel filtration never yielded homogeneous peaks. CHAPS (0.5%) could be used to desalt a homogenate containing alkylglycerol monooxygenase without losing its activity. For more sophisticated enrichment protocols however, 100% solubilisation as evaluated by appearance of a symmetrical peak in gel filtration was needed, which was never achieved. We therefore could not follow this strategy further. Also, alternative strategies such as functional library screens as well as total RNA injection screens in *Xenopus laevis* oocytes did not lead to a sequence for this enzyme.

We then chose to pursue bioinformatic strategies including homology searches to the known tetrahydrobiopterin-dependent enzymes to look for a possible imprint for cofactor binding as well as browsing motifs and expression data of open reading frames with unassigned function, and finally we expressed the ten most promising candidates in Chinese hamster ovary (CHO) cells. By this, we were finally able to identify formerly named transmembrane protein 195 (TMEM195) as the gene coding for alkylglycerol monooxygenase [14].

Alkylglycerol monooxygenase sequence analysis

The sequence of alkylglycerol monooxygenase revealed some very striking features, which can at least partly explain the difficulties in working with alkylglycerol monooxygenase protein. Within the sequence, there is a motif found which is denominated as fatty hydroxylases motif (PFAM04116). This motif can be found in very few other enzymes in our body, between these there are two better described ones, cholesterol-25-hydroxylase and fatty acid hydroxylase, as well as some still uncharacterised hydroxylases and desaturates. None of these members has ever been purified to homogeneity and they are thought to be so labile because of an 8-histidine conserved motif which is thought to bind an iron-oxygen-iron cluster that is crucial for catalysis [15, 16].

The alkylglycerol monooxygenase sequence turned out to lack detectable sequence homology to any other tetrahydrobiopterin-dependent enzyme and therefore forms a third and distinct class of tetrahydrobiopterin-dependent enzymes in addition to the aromatic amino acid hydroxylases and the nitric oxide synthases [14].

Hydrophobicity analysis of the sequence also provided evidence that this enzyme is highly hydrophobic with approximately half of its sequence buried in the membrane bilayer, therefore rendering it difficult to handle it without losing the activity. Bacterial expression of such a highly hydrophobic protein proved to be unachievable in its full length version. We therefore tried to express smaller fragments of alkylglycerol monooxygenase, which was partly successful; however, no activity could ever be obtained from these pieces (see a separate article in this issue by Mayer et al., Expression of full-length human alkylglycerol monooxygenase and fragments in *E. coli*).

Insights into alkylglycerol monooxygenase catalysis and structure

To obtain some insights into the catalytically important residues, we mutated the eight histidines contained in the conserved motif and expressed the mutant protein in CHO cells. All eight proved to be crucial for activity [14]. We extended this analysis to 26 further residues including additional

histidines, but also glutamates and aspartates, as well as glutamines and some other residues [17]. In the aromatic amino acid hydroxylases binding of tetrahydrobiopterin is mediated by a glutamate residue adjacent to one of the histidines binding the non-heme iron. When transfecting the mutants into CHO cells and assaying the cell homogenates for alkylglycerol monooxygenase activity, it became evident that there was indeed a glutamate next to one of the eight histidines forming the motif that, when mutated into alanine, led to an 18-fold increase of the Michaelis Menten constant for tetrahydrobiopterin. Furthermore, a ninth histidine was identified which is needed for catalysis [17].

Owing to the great difficulties in expressing this enzyme in bacteria, experimental data for its structure are not accessible at this time. We therefore tried ab initio modelling of residues 37–205 of human alkylglycerol monooxygenase using Rosetta membrane. The resulting structure together with four membrane prediction tools for the whole sequence led us to a first idea of how alkylglycerol monooxygenase is lying in the membrane. All residues being proved to be crucial for catalysis by site-directed mutagenesis nicely fold together yielding a proposed region for the active site of the enzyme (Figure 1).

Alkylglycerol metabolism and physiological implications

Alkylglycerol monooxygenase cleaves the ether bond in saturated ether lipids: the alkylglycerols. It is the only enzyme described which is able to perform this cleavage step, which is thought to occur through a tetrahydrobiopterin-dependent hydroxylation at the C atom adjacent to the ether bond and subsequent rearrangement of the resulting hemiacetal into the corresponding aldehyde and the glycerol derivative. The metabolic environment this catalytic step belongs to is the ether lipid metabolism, which includes biosynthetic routes for all ether bond containing lipids including the plasmalogens and platelet-activating factor, as well as the degradation of this species. Interestingly, many enzymes of these pathways including alkylglycerol monooxygenase have only recently been

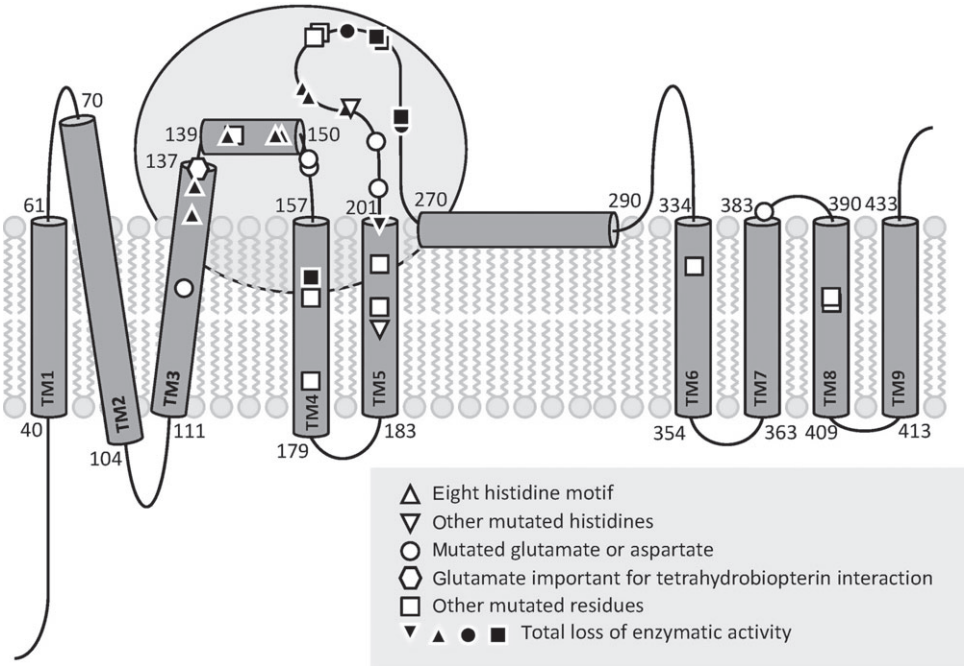

Figure 1 Schematic topography of alkylglycerol monooxygenase.
Ab initio modelling and prediction tools for transmembrane (TM) segments yielded this schematic depiction of how alkylglycerol monooxygenase might be folded into the membrane bilayer and where the active site of the enzyme is most likely to be located (highlighted in grey). Engineered mutations are shown by various symbols: triangles upward-pointing, eight histidines forming the fatty acid hydroxylases motif; triangles downward-pointing, further histidines; circles, glutamates and aspartates; squares, other mutated residues. The hexagon depicts the glutamate at position 137, which was shown to be crucial for the interaction with tetrahydrobiopterin. All mutations which led to a complete loss in enzymatic activity are shown in white frames and black filling. Reproduced, with permission, from [17]. © The Biochemical Society.

assigned a sequence or are still orphan enzymes with no sequence information available. For a more detailed description of the ether lipid metabolism and the related enzymes, the reader is referred to a recent review [18].

So far no physiological and pathophysiological roles of alkylglycerol monooxygenase have been reported. One way to deduce possible implications at this stage is to look at the roles of its substrates: the alkylglycerols in the body. These lipids are important in sperm development, they are structural components of the brain and a decrease in their levels makes the eye more prone to develop cataracts [19–22]. Furthermore, platelet-activating factor is an inflammatory signal mediator synthesised by cells involved in host defence. It plays a role in a diverse range of processes in the body including signalling in shock and sepsis, mediation of allergy, inflammation and bronchoconstriction (for a review, see [23]).

Influences of alkylglycerol monooxygenase on alkylglycerol levels might therefore be implicated with changes in sperm maturation, changes to brain and lens structure, and might also terminate the inflammatory signal mediated by platelet-activating factor. Experimental proof of this is still not available and is an important quest to be pursued in the future.

Conclusion

Alkylglycerol monooxygenase was first described in 1964 as a tetrahydrobiopterin-dependent enzyme that can cleave the ether bond in alkylglycerols [8]. After this, it took more than four decades to identify the sequence of this integral membrane protein [14] and to obtain some insights into its membrane disposition and structural and catalytic features [17]. Current work now focusses on the role of alkylglycerol monooxygenase in physiology and pathophysiology.

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References

1. Werner ER, Blau N, Thöny B. Tetrahydrobiopterin: biochemistry and pathophysiology. Biochem J 2011;438:397–414.
2. Kaufman S. The participation of tetra-hydrofolic acid in the enzymic conversion of phenylalanine to tyrosine. Biochim Biophys Acta 1958;27:428–9.
3. Nagatsu T, Levitt M, Udenfriend S. Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. J Biol Chem 1964;239:2910–7.
4. Lovenberg W, Jequier E, Sjoerdsma A. Tryptophan hydroxylation: measurement in pineal gland, brainstem, and carcinoid tumor. Science 1967;155:217–9.
5. Mayer B, John M, Heinzel B, Werner ER, Wachter H, Schultz G, et al. Brain nitric oxide synthase is a biopterin- and flavin-containing multi-functional oxido-reductase. FEBS Lett 1991;288:187–91.
6. Tayeh MA, Marletta MA. Macrophage oxidation of L-arginine to nitric oxide, nitrite, and nitrate. Tetrahydrobiopterin is required as a cofactor. J Biol Chem 1989;264:19654–8.
7. Palmer RM, Moncada S. A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. Biochem Biophys Res Commun 1989; 158:348–52.
8. Tietz A, Lindberg M, Kennedy E. A new pteridine-requiring enzyme system for the oxidation of glyceryl ethers. J Biol Chem 1964;239:4081–90.
9. Ishibashi T, Imai Y. Solubilization and partial characterization of alkylglycerol monooxygenase from rat liver microsomes. Eur J Biochem 1983;132:23–7.
10. Ishibashi T, Imai Y. Affinity purification of alkylglycerol monooxygenase from rat liver microsomes by chimyl alcohol-Sepharose 4B column chromatography. J Lipid Res 1985;26:393–5.
11. Kötting J, Unger C, Eibl H. Substrate specificity of O-alkylglycerol monooxygenase (E.C. 1.14.16.5), solubilized from rat liver microsomes. Lipids 1987;22:831–5.
12. Taguchi H, Armarego WL. Glyceryl-ether monooxygenase [EC 1.14.16.5]. A microsomal enzyme of ether lipid metabolism. Med Res Rev 1998;18:43–89.
13. Werner ER, Hermetter A, Prast H, Golderer G, Werner-Felmayer G. Widespread occurrence of glyceryl ether monooxygenase activity in rat tissues detected by a novel assay. J Lipid Res 2007;48:1422–7.
14. Watschinger K, Keller MA, Golderer G, Hermann M, Maglione M, Sarg B, et al. Identification of the gene encoding alkylglycerol monooxygenase defines a third class of tetrahydrobiopterin-dependent enzymes. Proc Natl Acad Sci USA 2010;107:13672–7.
15. Shanklin J, Whittle E, Fox BG. Eight histidine residues are catalytically essential in a membrane-associated iron enzyme, stearoyl-CoA desaturase, and are conserved in alkane hydroxylase and xylene monooxygenase. Biochemistry 1994;33:12787–94.
16. Shanklin J, Guy JE, Mishra G, Lindqvist Y. Desaturases: emerging models for understanding functional diversification of diiron-containing enzymes. J Biol Chem 2009;284:18559–63.
17. Watschinger K, Fuchs JE, Yarov-Yarovoy V, Keller MA, Golderer G, Hermetter A, et al. Catalytic residues and a predicted

structure of tetrahydrobiopterin-dependent alkylglycerol mono-oxygenase. Biochem J 2012;443:279–86.
18. Watschinger K, Werner ER. Orphan enzymes in ether lipid metabolism. Biochimie 2013;95:59–65.
19. Rodemer C, Thai T, Brugger B, Kaercher T, Werner H, Nave K, et al. Inactivation of ether lipid biosynthesis causes male infertility, defects in eye development and optic nerve hypoplasia in mice. Hum Mol Genet 2003;12:1881–95.
20. Gorgas K, Teigler A, Komljenovic D, Just WW. The ether lipid-deficient mouse: tracking down plasmalogen functions. Biochim Biophys Acta 2006;1763:1511–26.
21. Komljenovic D, Sandhoff R, Teigler A, Heid H, Just W, Gorgas K. Disruption of blood-testis barrier dynamics in ether-lipid-deficient mice. Cell Tissue Res 2009;337:281–99.
22. Teigler A, Komljenovic D, Draguhn A, Gorgas K, Just WW. Defects in myelination, paranode organization and Purkinje cell innervation in the ether lipid-deficient mouse cerebellum. Hum Mol Genet 2009;18:1897–908.
23. Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM. Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. Crit Rev Clin Lab Sci 2003;40:643–72.