Crystal structure of the *Saccharomyces cerevisiae* monoglyceride lipase Yju3p

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**Abstract**

Monoglyceride lipases (MGLs) are a group of α/β-hydrolases that catalyze the hydrolysis of monoglycerides (MGs) into free fatty acids and glycerol. This reaction serves different physiological functions, namely in the last step of phospholipid and triglyceride degradation, in mammalian endocannabinoid and arachidonic acid metabolism, and in detoxification processes in microbes. Previous crystal structures of MGLs from humans and bacteria revealed conformational plasticity in the cap region of this protein and gave insight into substrate binding. In this study, we present the structure of a MGL from *Saccharomyces cerevisiae* called Yju3p in its free form and in complex with a covalently bound substrate analog mimicking the tetrahedral intermediate of MG hydrolysis. These structures reveal a high conservation of the overall shape of the MGL cap region and also provide evidence for conformational changes in the cap of Yju3p. The complex structure reveals that, despite the high structural similarity, Yju3p seems to have an additional opening to the substrate binding pocket at a different position compared to human and bacterial MGL. Substrate specificities towards MGs with saturated and unsaturated alkyl chains of different lengths were tested and revealed highest activity towards MG containing a C18:1 fatty acid.

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1. Introduction

Lipases are hydrolases that cleave ester bonds in lipids and therefore act at a water–lipid interface. Many lipases contain a variable lid or cap region which covers the active site and forms the substrate binding pocket in combination with an α/β-hydrolase core domain. In some lipases, this cap region is considered to play a role in interfacial activation [1,2]. Monoglyceride lipases (MGLs) are a subclass of lipases and catalyze the breakdown of monoglycerides (MGs) resulting in free fatty acids and glycerol. MGLs are predominantly specific towards MGs, yet are not reported to be regiospecific or stereospecific [1, 3, 2–3, 3–5]. MGLs are conserved across all species and serve different biological roles in different species and tissues. In mammals, MGLs play an essential role in energy homeostasis, where they catalyze the last step of phospholipid and triglyceride breakdown [10]. Furthermore, they play a role in lipid signaling by regulating the levels of the endocannabinoid 2-arachidonoyl-glycerol (2-AG), the most abundant endogenous agonist of cannabinoid receptors [11–13]. By hydrolyzing 2-AG, MGL also determines the availability of arachidonic acid for prostaglandin synthesis in the brain [14]. In microbes, MGs have been shown to be toxic rendering MGL important for detoxification processes [15–17]. Pathogenic bacteria, including *Mycobacterium tuberculosis*, accumulate lipids in the dormant phase. Enzymes involved in degradation of these lipid depots may be essential in providing energy and precursors for cell wall synthesis during the reactivation step and the chronic phase [18]. Due to these different biological roles, MGLs are interesting subjects for drug targeting [14, 18–20].

Despite the fact that MGLs have been studied for decades, very little three-dimensional (3D) structural data is available. The only experimentally determined 3D structures are those of human MGL (hMGL) and MGL from *Bacillus* sp. H-257 (bMGL) [12, 21–25]. bMGL was determined in its free form and in complex with inhibitors, bMGL in its free form and in complex with inhibitors and the natural substrate. The structures of the two enzymes are very similar despite the large evolutionary distance between bacteria and humans. Apart from the

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conserved α/β-hydrolase core, two additional features are especially remarkable, namely the similarity of the overall shape of the cap regions and its conformational flexibility [22,23]. Based on the conserved overall shape of the cap region in hMGL and bMGL, we hypothesized that this general cap architecture might also be present in other species. In order to test this, we herein focus on Yju3p, the MGL ortholog from Saccharomyces cerevisiae [26,27]. Low sequence identity to bMGL and hMGL precludes reliable homology modeling without introducing a heavy model bias, rendering an experimentally determined structure a key requirement. A solubility enhancing mutation was introduced into the sequence of Yju3p (hereafter named s-Yju3p) which allowed concentrations of the purified protein in the mg/ml range without the addition of detergents. Furthermore, we investigated whether the modes of substrate binding are comparable to MGLs with known structures by analyzing complex structures mimicking the tetrahedral reaction intermediate. Thus, we present crystal structures of s-Yju3p in its free form and in complex with an inhibitor mimicking the tetrahedral intermediate of a C20:0 MG during hydrolysis. s-Yju3p harbors an α/β-hydrolase core and a cap region similar to those of hMGL and bMGL. Interestingly, the structure of the inhibitor complex revealed differences in the mode of substrate binding in s-Yju3p compared to the other two MGLs. Analysis of the MG hydrolase activity of s-Yju3p unveiled differences in substrate preferences with respect to the saturation state of the MG substrate. These data can be rationalized by differences in the shapes of the active site cavities in the three MGLs.

2. Experimental methods

2.1. Synthesis of substrate analogs

p-Nitrophenyl esters of alkyl phosphonic acids were used to mimic the natural MG substrate and employed for co-crystallization studies. The C20:0 MG mimicking substrate analog was synthesized as described previously [22]. The identity of the compound was confirmed using mass spectrometry and NMR spectroscopy. As outlined before, the substrate analog contained a carbon chain consisting of only 18 carbons, yet it mimics a C20:0 substrate. This can be rationalized by its chemical structure which has a phosphor-atom at the position where the carbon of the carboxyl group would be placed and an oxygen-atom is at the position of the first methylene group of the carbon chain (Fig. 1E).

2.2. Cloning and mutagenesis of s-Yju3p

A solubility variant Leu175Ser was produced by applying QuikChange™ (Agilent Technologies) site directed mutagenesis to the...
yu3p gene according to the manufactures instructions using the follow-
ing primers: forward primer: 5′-GGC GAA ATT TTC ACC AAC GGT GAT GAT CCA CAC TGG-3′; reverse: 5′-CCA GTG TCG ATC CTT ACC CTT GGT GAA AAT TTC GCC-3′. It should be noted here that, the variant was described previously as active in hydrolyzing MGs [26], ii) the amino acid position is not conserved when comparing the sequence with hMGl or mouse MGL and, iii) the amino acid is placed in a surface exposed position of the core domain and thus is very unlikely to interfere with the fold of the protein. The resulting variant is called s-Yju3p throughout the re-

2.3. Expression and purification

s-Yju3p was expressed and purified as described previously [27]. Ex-
pression and affinity chromatography purification for hMGl and Yju3p were done using the same protocol and buffers as for s-Yju3p with the exception that the lysis buffer contained 0.5% IGEPAh CA-630 and the wash and elution buffers 0.05% IGEPAh CA-630, respectively. bMGL was purified as described previously [22]. To compare the activities of the lipases, a buffer containing 0.05% IGEPAh was used for all proteins.

2.4. Monoglyceride hydrolase activity assay

Since MGLs are not regioselective (Fig. 1A) and the 1(3)-racemic monoglyceride (1(3)-rac MG) substrates are commercially available with different chain lengths and saturations, we used 1 (3)-rac MGs as substrates. The monoglyceride hydrolase activity assays for Yju3p, s-

2.5. Crystallization and data collection: s-Yju3p in free form and in complex with a MG C20:0 analog

Crystals of s-Yju3p in its free form were obtained from a protein solu-
tion at 14 mg/mL and a reservoir solution containing 0.1 M Bicine/Trizma base pH 8.7, 10% w/v PEG 20 000, 20% v/v PEG MME 550 and 0.03 M sodium nitrate, 0.03 M disodium hydrogen phosphate, and 0.03 M am-

2.6. Heavy metal soaking and MAD data collection

s-Yju3p crystals were harvested and transferred to an extra drop consisting of 1.5 μL of a 10 mM K2Pt(NO3)4 solution and 2.5 μL of the

2.7. Data processing and structure refinement: s-Yju3p

The three data sets collected for crystals soaked with K2Pt(NO3)4 were indexed and integrated using XDS [30] and scaled using Scala [31]. These data sets were then uploaded to the Auto-Rickshaw server [32]. A native data set without heavy atom derivatives with a resolution of 2.5 Å measured at SLS (Villigen, Switzerland) was indexed and inte-

2.8. Data processing and structure refinement: s-Yju3p substrate analog complex

Diffraction data from a crystal of the s-Yju3p C20:0 MG analog complex were indexed and integrated using iMosflm [37] and scaled using Scala [31]. 5% of the reflections were held back after data redu-
tion in order to calculate Rfree values for evaluation of the refinement quality. The resolution was cut at 2.5 Å in order to achieve acceptable Rmerge values. The refinement was carried out using Coot [35] and ph

2.9. Molecular dynamics simulation

pKa values and protonation states of the titratable amino acids at pH 7 were calculated using TITRA employing the Tanford–Kirkwood
3. Results and discussion

3.1. Engineering of s-Yju3p, a solubility enhanced variant of Yju3p

Previous reports identified Yju3p as a membrane-bound or lipid droplet associated protein [44]. Accordingly, purification and concentration of recombinant Yju3p required the presence of detergents in order to prevent aggregation (e.g. NP-40, Mega8) [26]. Therefore, we used a solubility enhancement strategy as described for hMGL to identify potential hydrophobic residues in the cap region which might be involved in aggregation [24]. Employing this strategy, hMGL carrying the variations Leu169Ser, Leu176Ser, and Lys36Ala had yielded crystals diffracting to 1.35 Å. Based on a sequence alignment, we assumed that amino acid Leu175 of Yju3p corresponds to Leu169 in hMGL. Indeed, introducing a Leu175Ser mutation proved successful and allowed purification, concentration and crystallization of the protein even in the absence of detergents. MG hydrolase assays verified that the soluble s-Yju3p variant is still active against MGs in the mmol/(hour*mg protein) range (Fig. 1A).

3.2. The 3D structure of s-Yju3p

s-Yju3p crystallized in space group P2₁2₁2₁. The structure was determined using a combination of multi-wavelength anomalous dispersion (MAD) and molecular replacement using the fragments obtained during model building after MAD. We observe four molecules in the asymmetric unit. The PISA server [45] suggests a tetramer to be stable, yet we observed a peak corresponding to the size of monomeric protein in the size exclusion chromatogram. The overall structure of s-Yju3p harbors an α/β-hydrolase core and a cap containing four helices of different lengths (Fig. 1B, C). The core harbors the active site with the catalytic triad residues Ser123, Asp251 and His281 as the nucleophile attacking the partially positively charged carbonyl carbon of the substrate. Comparison with bMGL and hMGL structures suggests that the oxyanion hole is formed by the main chain amide groups of Met124 and Phe49. The cap is located around the catalytic center in a position that could facilitate substrate binding (Fig. 1B–D). In the N-terminal region of the cap, the short Helix 1 (Pro156–Asn161) and the longer Helix 2 (Thr164–Phe174) are just separated by Lys162 and Pro163, which induce a bent in the orientation of the catalytic center.

| Table 1 | Processing and refinement statistics of s-Yju3p structures. |
|---------|---------------------------------------------------------------|
|         | s-Yju3p free SLS                                             | s-Yju3p C20:0 analog complex ID-29 ESRF |
| Data collection | Wavelength | 0.999900 Å | 0.97939 Å |
| Resolution | 45.27 Å–2.49 Å (2.57 Å–2.49 Å) | 82.72 Å–2.5 Å (2.64 Å–2.5 Å) |
| Space group | P2₁2₁2₁ | P2₁2₁2₁ |
| Unit cell | a b c | a b c |
| Total no. of reflections | 439,569 | 215,498 |
| Unique reflections | 49,134 | 47,973 |
| Rmerge | 0.059 (1.410) | 0.052 (0.965) |
| Rpim | 0.022 (0.536) | 0.024 (0.455) |
| Rfree | 0.065 (1.597) | 0.052 (0.965) |
| CC 1/2 | 0.996 (0.49) | 0.997 (0.178) |
| Ramachandran disallowed | 0.771° | 0.806° |
| Multiplicity | | |
| Solvent atoms | 408 | 151 |
| Model geometry | | |
| r.m.s.d. bonds | 0.005 Å | 0.004 Å |
| r.m.s.d. angles | 0.771° | 0.806° |
| Ramachandran distribution | | |
| Ramachandran favored | 96.1% | 96.91% |
| Ramachandran disallowed | 0.16% | 0.33% |
| Rotation outlier | 0.75% | 0.95% |

To calculate Rmerge, 5% of the reflections were excluded from the refinement. Rmerge is defined as $R_{merge} = \frac{\sum_{j=1}^{n} \sum_{hkl} |I_{hkl,j} - \langle I_{hkl,j} \rangle|}{\sum_{j=1}^{n} \sum_{hkl} |I_{hkl,j}|}$. Rfree is defined as $R_{free} = \frac{\sum_{j=1}^{n} \sum_{hkl} |I_{hkl,j} - \langle I_{hkl,j} \rangle|}{\sum_{j=1}^{n} \sum_{hkl} |\langle I_{hkl,j} \rangle|}$. Data in parentheses correspond to the highest resolution shell. r.m.s.d., root mean square deviation. The CC 1/2 is the Pearson correlation coefficient between average intensities of two half data sets [49].
the short Helices 3 (Leu186–Ile189) and 4 (Lys193–Ser201) at the opposite end of the cap. Residues Arg179–Asp181 and Tyr209–Ser211 are arranged in an antiparallel manner and bring the forth and back leg of the cap in spatial proximity (Fig. 1C). Arg264, which is a glutamine in the native sequence, is located in the cap region in a loop near the end of Helix 2 (Fig. 1B). This spatial position corresponds nicely to the position of Leu169Ser near the end of the first helix in hMGL which served as template for our solubility enhancement design. Three small holes were observed in the surface of chain D, yet none of them seems big enough to fit the glycerol head group of an MG substrate or our MG-analog in the presence of a p-nitrophenyl group.

3.3. s-Yju3p tetrahedral intermediate analog complex

Crystals diffracting to 2.2 Å were obtained from the co-crystallization setup with a p-nitrophenyl ester of alkyl phosphonic acid mimicking a C20:0 MG analog (Fig. 1E). The crystals were isomorphous to the crystals of the free form of s-Yju3p. Additional electron density could be observed near the active site in chains A, B and D; though it was not unambiguous enough to be interpreted as the C20:0 MG analog. In chain C however, we could observe electron density resembling the shape of our ligand (Fig. 1F). In some regions of the ligand carbon chain, the electron density was poorly defined. This might be caused by a low occupancy of the ligand (e.g. due to low water solubility) or by the flexibility of the carbon chain. The substrate analog oxygen that is equivalent to the carbonyl oxygen of the substrate is stabilized by hydrogen bonds from the backbone nitrogen of the oxyanion-hole residues Met124 and Phe49 (Fig. 1E, G). The substrate entrance channel is mostly outlined by hydrophobic side chains (Leu151, Leu154, Thr158, Met159, Lys162, Gln165 and Thr182 from the cap and Phe49, Ile215, Phe218, Met219, and Ile253 from the core) (Fig. 1H, I). This hydrophobicity might be necessary to accommodate the hydrophobic carbon chain of the MG substrate in the binding pocket. Met159 and Ile166 of the cap line the outside of the entrance channel and form hydrophobic interactions with the carbon chain of the substrate analog partially sticking out into the aqueous environment (Figs. 1H, I, 2) [46]. Unexpectedly, carbon-atoms C12 to C18 (corresponding to C14 to C20 in an actual MG substrate) stick out of the hydrophobic substrate binding channel between the cap and the core regions of the lipase (Fig. 2B). This opening is quite distinct from the bMGL structures in the open conformation and from the substrate entrance tunnels observed in complex structures of bMGL with different ligands [22,23,25]. The physiological
relevance of this wedge-like opening observed in presence of the covalently bound ligand still needs to be established. Crystals of the free form and the tetrahedral intermediate mimicking complex were isomorphous and the packing was identical. Therefore, we conclude that the observed conformational changes are not caused by crystal contacts in the cap region but represent an intrinsic conformational rearrangement.

3.4. Conformational flexibility in the cap of s-Yju3p

Crystal structures of hMGL and bMGL revealed conformational flexibility of the cap region. The experimental structures of free s-Yju3p and of the lipase in complex with the covalently bound inhibitor (Fig. 2A, B) also pointed towards conformational flexibility of the s-Yju3p cap. Three of the four chains in the free form structure showed a closed surface with only one small hole in chain D (Fig. 2A). If this observed closed cap conformation were to be rigid, such an enzyme could not be active since it does not provide a possibility for the substrate to reach the active site. Thus we hypothesized that additional cap conformations of s-Yju3p have to exist. Indeed, slight conformational changes indicating breathing movements of the lipase could be observed in one chain of free s-Yju3p. The structure of s-Yju3p mimicking the tetrahedral reaction intermediate in complex with the C20:0 MG substrate analog provided further proof for flexibility of the cap (Fig. 2B, C). In chains A, B and D, the cap region adopted the same conformation as in the free form structure. In chain C conformational flexibility could be observed especially in Helix 2 of the cap. Residues Thr164–Ile167 show marked displacement of the Cα atoms compared to the position observed in the free form (Cα-Cα distances of 2.8, 3.0, 2.4, and 2.9 Å, respectively) while the rmsd value of the cap region encompassing residues Pro156–Phe174 is 1.3 Å (Fig. 2C). In this region, a completely connected electron-density for the protein chain of free s-Yju3p can be observed which is clearly different in the s-Yju3p complex structure (Fig. S1).

Upon this conformational movement, the distance between the side chains of Ile166 (located in Helix 2 of the cap) and Met159 (located in Helix 1 of the cap) is increased from 3.9 Å to 7.4 Å (Fig. 2C-E). In the complex structure, this hole is occupied by the carbon chain of the C20:0 MG substrate analog (Fig. 2B, F). The published ligand bound structures of hMGL do not contain a natural MG substrate or a substrate analog. Nevertheless, the structure of hMGL in complex with a molecule termed ‘compound 1’ also showed conformational rearrangements of the cap (PDB 3PE6, (24)). Different cap conformations were observed for bMGL which appeared to arise from stochastic fluctuations or enzyme breathing rather than being ligand induced [22,23,25,26].

In analogy, we assume stochastic fluctuations in the cap of s-Yju3p could facilitate entry of the substrate to the active site deeply buried within the protein. In order to get first clues on such a predicted open conformation of s-Yju3p, four 100 ns molecular dynamics simulations of the protein solvated in explicit water starting from the closed conformation were carried out. In all four simulations, the cap region (from residue Ile153 to Phe212) showed a significantly higher flexibility than the protein core visible in the RMSF per residue plot (Fig. 3A).

In two simulation runs the protein stayed in a rather closed state, whereas a significant opening leading to an open conformation could be observed in two different simulations. The overall back bone rmsd reached a plateau around 2.0 Å, the rmsd for the α/β-hydrolase core only stayed below 1.8 Å. The cap region however, reached a maximum rmsd of 5.1 Å after approximately 22 ns, before the protein was again morphing to a closed conformation (Fig. 3B). In contrast to an unfolding of the cap region during the simulation, the observed increase and subsequent decrease of the cap rmsd (relative to the equilibrated closed conformation) is a strong indication for a directed cap movement from the closed to the open state (and back). In this open state, the active site entrance was visible and the active site Ser123 was accessible from the solvent (Fig. 3C, D). It should be noted here, that the position of the opening observed in the MD simulations is different to the one observed in our crystal structure with the C20:0 MG substrate analog exiting the

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**Fig. 3.** s-Yju3p molecular dynamics simulation. A, RMSF (root mean square fluctuation) in Å against amino acid residue. B, RMSD (root mean square deviation) in Å against molecular dynamics simulation time, black shows the RMSD for the entire protein backbone, green shows the RMSD only for the backbone of the α/β-hydrolase core, red shows the RMSD only for the backbone of the cap region. C-E: surface representation of s-Yju3p during MD-simulation in standard view (C) and top-view (D) at 0 ns and after 25 ns in top view (E). The s-Yju3p α/β-hydrolase core and the cap are shown in light blue and beige, respectively. E, the active site serine depicted in red is accessible in the open conformation.
substrate binding pocket between the cap and the core domain (see also Fig. 5A, B). In conclusion, the MD simulations strongly suggest that Yju3p is capable of showing an open conformation similar to the ones in hMGL and bMGL.

3.5. Cavity shape and substrate binding versus substrate selectivity

Comparison of the cap regions of s-Yju3p and the other structurally characterized MGLs (hMGL and bMGL) shows high conservation of the overall S-shape of the cap region across different species. Despite the differences in secondary structure elements, the overall course of the protein backbones in these three different MGLs is very similar (Fig. 4A–C) [12,21–24]. It could even be argued, that the interrupted cap helix in Yju3p represents an evolutionary necessary functional intermediate when comparing MGLs from Bacillus, S. cerevisae and Homo sapiens. The introduction of the amphipathic cap helix apparently changes the intracellular localization of the MGLs from a water-soluble, cytosolic to a membrane- and LD-associated lipase. Consequently, the

Fig. 4. The general cap architecture is conserved among MGLs from different species. A, cap region of s-Yju3p C20:0 analog complex (PDB code 4ZXF); B, cap region of bMGL (PDB code 4KE8); C, cap region of hMGL (PDB code 3HJU). All panels are in top-view orientation.

Fig. 5. Substrate accessibilities and substrate specificities of MGLs from S. cerevisae, Bacillus sp. H-257, and Homo sapiens. Panels A–D and H–J are depicted in side-view orientation. A–D, active site serines are marked with arrows, entrances are highlighted with red circles, cavities inside the proteins are shown as gray surfaces. A, s-Yju3p in complex with the C20:0 substrate analog (cap region in orange and the α/β-hydrolase core in green); the cavity is shown after removal of the ligand (PDB code 4ZXF). B, open conformation of s-Yju3p resulting from the MD simulation (cap region in red and the α/β-hydrolase core in orange). C, bMGL (PDB code 4KE8, cap region in pink, α/β-hydrolase core in purple). D, hMGL (PDB code 3HJU, cap region in light blue and α/β-hydrolase core in blue). E–G, selectivity of different MGLs for saturated and unsaturated MG substrates. Saturated substrates are to the left of the dashed line and unsaturated to the right. MG hydrolase activity assay with MG substrates containing different fatty acids for Yju3p (E) bMGL (F), and hMGL (G). H, substrate binding pocket of s-Yju3p (C20:0 analog complex) (blue surface) with the bound C20:0 ligand as blue sticks. I, substrate binding pocket (yellow surface) of bMGL (PDB code 4KE8) and the bound C16:0 analog as yellow sticks. J, substrate binding pocket of hMGL (PDB code 4UQJ) as green surface, the bound inhibitor SAR127303 is displayed in stick representation.
recruitment of different substrates from different cellular compartments became also possible.

s-Yju3p seems to have a substrate binding mode distinct from bMGL and hMGL. In s-Yju3p, the carbon chain of the substrate analog appears to be able to act as a wedge between the α/β-hydrolase core and the cap region embedded by Leu166 and Met159 (Fig. 5A, red circle). Thus, the end of the alkyl-chain in the complex structure of s-Yju3p is observed below cap Helices 1 and 2. The path from the active site serine to the opening of the binding pocket of hMGL and bMGL follow a straight line and suggest positioning of the substrate above the corresponding helices (Fig. 5A–D). The transition-state intermediate of s-Yju3p was crystallized with a ligand mimicking a C20:0 alkyl chain. One might assume that this conformation is linked to the preference of this long aliphatic chain to be placed in a bent form in the hydrophobic substrate binding pocket. A more direct, straight path would place larger parts of the alkyl chain into the hydrophilic environment outside the binding pocket. Interestingly, MD simulations also suggest a possible open conformation with an opening between the first stretch (Helices 1 and 2) and the second stretch (Helix 3) in the cap region. This is similar to the open conformations experimentally observed in bMGL and hMGL with the substrate entrance channel within the first turn of the S-shape within the cap region (Fig. 5C–D, red circles).

In this study, we also determined specific activities of Yju3p, hMGL and bMGL with saturated and mono-unsaturated MGs of different chain lengths. MG hydrolase activity assays showed that Yju3p activity gradually decreased with increasing chain length using saturated MGs as substrate. Actually, we could not detect activity using 18:0 MG as substrate (Fig. 5E). This trend was also observed using saturated MGs for hMGL and bMGL (Fig. 5F, G), although these enzymes exhibited detectable activity against 18:0 MG. One might speculate that the ability to fit and enclose the alkyl chain within the binding pocket plays a role [22, 23]. Notably, Yju3p showed highest activity in the presence of monounsaturated MGs C16:1 and C18:1. The different shapes of the cavities might also influence substrate specificities in different organisms (Fig. 5H–J). The substrate preference of Yju3p nicely correlates to the most abundant fatty acids within yeast [47]. This preference of Yju3p for unsaturated long-chain fatty acids as substrates might be facilitated by the bend observed in the Yju3p cavity (Fig. 5H). A saturated fatty acid should also be able to form a bend in the carbon chain, yet the preformed bend in an unsaturated fatty acid might preferably select for accommodation of a long aliphatic chain inside the protein. hMGL and bMGL exhibit highest activity using C12:0 as substrate. MGs with short alkyl chain length are toxic to bacteria which can explain the necessity of bacterial MGL to be extremely efficient in the turn-over of these substrates. bMGL does not differentiate between C16:0 and C16:1 but shows different activity for C18:0 and C18:1 MG substrates. This might be explained by the fact that longer carbon chains need to be bend to be accommodated in the substrate binding pockets while a unsaturated substrate is already pre-bent and easier to accommodate [22] (Fig. 5H–J). hMGL shows less selectivity between C18:0 and C18:1 than Yju3p and bMGL. This could be caused by the broad substrate entrance channel of hMGL which applies less restriction on the rotamers of the fatty acid moiety that could be fitted inside the protein. It cannot be ruled out completely, that the physico-chemical properties of the substrates and concomitant different modes of substrate presentation in the used aqueous micelle systems also influence the measured absolute activities in the assay.

Although the overall shape of the cap in MGLs seems to be conserved across species, we could show that there are differences in the details of the cap structures, substrate binding pockets, and in the substrate preference of Yju3p compared to hMGL and bMGL. While the structural differences observed fit the differences in substrate preference and provide a basis for their explanation, further biochemical data including enzyme kinetics, on- and off-rates, etc. are needed in order to get a clear understanding of the mechanisms underlying the substrate selectivity in MGLs.

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Author contributions

Conceived and designed the experiments: PA SR RB RBG RZ MO. Performed the experiments: PA SR JL MS NM KMPD CCG. Analyzed the data: PA SR RZ KG CCG RZ MO. Wrote the paper: PA SR CC CG MO.

Conflict of interest

The authors declare that they have no conflict of interest with the contents of this article.

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