Structural and kinetic insights into flavin-containing monooxygenase and calponin-homology domains in human MICAL3

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MICAL is an oxidoreductase that participates in cytoskeleton reorganization via actin disassembly in the presence of NADPH. Although three MICALs (MICAL1, MICAL2 and MICAL3) have been identified in mammals, only the structure of mouse MICAL1 has been reported. Here, the first crystal structure of human MICAL3, which contains the flavin-containing monooxygenase (FMO) and calponin-homology (CH) domains, is reported. MICAL3 has an FAD/NADP-binding Rossmann-fold domain for monooxygenase activity like MICAL1. The FMO and CH domains of both MICAL3 and MICAL1 are highly similar in structure, but superimposition of the two structures shows a different relative position of the CH domain in the asymmetric unit. Based on kinetic analyses, the catalytic efficiency of MICAL3 dramatically increased on adding F-actin only when the CH domain was available. However, this did not occur when two residues, Glu213 and Arg530, were mutated in the FMO and CH domains, respectively. Overall, MICAL3 is structurally highly similar to MICAL1, which suggests that they may adopt the same catalytic mechanism, but the difference in the relative position of the CH domain produces a difference in F-actin substrate specificity.

1. Introduction

Flavin-dependent monooxygenases catalyze a variety of oxygenation reactions, including regioselective, chemoselective and stereoselective oxidation reactions, which can be accomplished by single oxygen transfer to target substrates (Hung et al., 2010, 2011). These enzymes are involved in several metabolic processes, including the biosynthesis of hormones and vitamins, the inactivation of signaling molecules, the excretion of xenobiotic substrates and the guidance of axons (Kaya et al., 2015; Drazic & Winter, 2014; Lee et al., 2013; Nadella et al., 2005). With regard to the regulation of axon guidance, the molecule interacting with CasL (MICAL) protein, which belongs to the flavin-dependent monooxygenase family, has recently received attention for its unique function in controlling actin assembly in conjunction with MsrB1 (Lee et al., 2013). MICAL is a multi-domain enzyme that is highly conserved across most animal kingdoms and participates in actin cytoskeletal reorganization (Kolk &
It has been functionally characterized to possess stereoselective methionine-oxidation activity for actin disassembly in mice and fruit flies. Its flavin-containing monooxygenase (FMO) domain oxidizes the two conserved methionine residues of actin to methionine-R-sulfoxides, while the calponin-homology (CH) domain is involved in actin binding, thereby enhancing the catalytic activity of the FMO domain (Gimona et al., 2002).

Three MICALs, MICAL1, MICAL2 and MICAL3, have been identified in mammals. They contain FMO and CH domains and share a common function of F-actin disassembly. In addition, MICAL2 and MICAL3 have been reported to localize to the nucleus, but MICAL1 is cytosolic (Lundquist et al., 2014; Fischer et al., 2005). Furthermore, MICAL1 is autoinhibited by its C-terminal coiled-coil domain, but MICAL2 is constitutively active for the regulation of actin stress fibers (Giridharan & Caplan, 2014). MsrB then reduces R-sulfoxides back to methionines. After this redox change, the actin monomer can be reassembled into an F-actin polymer, thereby conferring cytoskeletal reorganization (Lee et al., 2013). These cytoskeletal alterations regulate multiple cellular events including cell morphology and axon growth, and exocytosis in various tissues. However, the underlying mechanism of this regulation by MICAL proteins is poorly understood (Wu et al., 2018). The structure of mouse MICAL1 has been determined, which has helped to provide an understanding of how it functions at the molecular level (Siebold et al., 2005; Nadella et al., 2005; Sun et al., 2006). However, the structures of MICAL2 and MICAL3 have yet to be determined and thus the lack of structural comparison among mammalian MICALs has limited our understanding of both the shared and unique characteristics of these proteins. Here, we report the first crystal structure of human MICAL3, which contains the FMO and CH domains, at 1.9 Å resolution. Based on a comparison of human MICAL3 with mouse MICAL1 (PDB entry 4txi; Alqassim et al., 2016), we present their structural similarities and differences, as well as their kinetics, which depend on the interactions of the FMO domain with the CH domain.

2. Materials and methods

2.1. Cloning of human MICAL1 and MICAL3

The DNA regions encoding the FMO and CH domains of MICAL3 (hMICAL3FMOCH; amino-acid residues 1–700), as well as the FMO domain of MICAL3 (hMICAL3FMO; amino-acid residues 1–494), were amplified from human cDNA. Truncated forms of MICAL1 comprising the FMO and CH domains (hMICAL1FMOCH; amino-acid residues 1–616) and the FMO domain (hMICAL1FMO; amino-acid residues 1–489) were amplified from human MICAL1 cDNA purchased from the Korean Human Gene Bank. The oligonucleotide primers used for amplification can be found in Supplementary Table S1. After PCR amplification, hMICAL3FMOCH was cloned into the pET-28b plasmid (Novagen) using the restriction enzymes BamHI and XhoI. Other MICAL forms were digested with the same enzymes and inserted into the pET-28a vector. E213G and R350G substitutions were introduced into hMICAL3FMOCH in pET-28b by site-directed mutagenesis (hMICAL3FMOCHΔE213G,R530G; Haque et al., 2018). To enhance the solubility of the recombinant MICAL forms, they were transformed and overexpressed in the Escherichia coli Rosetta 2 pLysS strain (Novagen). The cells were grown at 37°C to an optical density at 600 nm (OD600) of approximately 0.6 in LB medium and were induced at 18°C with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). After 18 h, the cells were harvested by centrifugation at 2300g for 1 h and stored at −20°C.

2.2. Protein purification

For each MICAL form, the harvested cells were lysed by sonication in a buffer consisting of 50 mM Tris pH 8.5, 150 mM NaCl, 2 mM β-mercaptoethanol (Hwang et al., 2018). After centrifugation at 13 000 rev min−1 for 1 h at 4°C, the supernatant was loaded onto an Ni–NTA column (GE Healthcare) equilibrated with binding buffer (50 mM Tris pH 8.5, 150 mM NaCl, 2 mM β-mercaptoethanol) and eluted with elution buffer (50 mM Tris, 150 mM NaCl, 2 mM β-mercaptoethanol, 1 M imidazole). The purified MICAL proteins (except for hMICAL3FMOCH) were concentrated using Amicon ultra-centrifugal filters (Ultracel-30, 30 kDa cutoff; Millipore) and stored at −80°C. For the subsequent crystallization experiments, hMICAL3FMOCH was concentrated and further purified by gel-filtration chromatography on a Superdex S200 column (GE Healthcare) using elution buffer consisting of 50 mM Tris pH 8.5, 100 mM NaCl, 1 mM 1,4-dithiothreitol, 1% glycerol. The predicted molecular weight of hMICAL3FMOCH is ~79.4 kDa. The monomeric form identified in some fractions during gel-filtration chromatography was collected, concentrated to 25 mg ml−1 using Amicon ultracentrifugal filters and stored at −80°C.

2.3. Crystallization

Crystals were grown by sitting-drop vapor-diffusion screening at 293 K using the MCGS-1, MCGS-2, MCGS-3 and MCGS-4 screening kits (Anatrace) on an Intelli-Plate 96 (Hampton Research), in which 0.5 µl human MICAL3 solution was mixed with an equal volume of screening solution. The initial crystallization condition was 0.1 M Bicine–NaOH pH 9.0, 10% (v/v) (±)-2-methyl-2,4-pentanediol (MPD) (Hampton Research). For crystal optimization, we employed the hanging-drop vapor-diffusion method in a 24-well VDX plate (Hampton Research). Crystals were optimized by growth in 0.1 M Bicine–NaOH pH 9.2, 7% (v/v) MPD for one day. For cryoprotection, crystals were transferred into a
reservoir solution containing 20% (v/v) glycerol before flash-cooling in liquid nitrogen.

2.4. Structure determination

A diffraction data set was collected on beamline 5A at Pohang Accelerator Laboratory, Republic of Korea at a wavelength of 0.9794 Å and the data were processed and scaled using SCALEPACK and DENZO from the HKL-2000 program package (Otwinowski & Minor, 1997). The crystal belonged to space group $P_2_1$, with unit-cell parameters $a = 65.233$, $b = 94.363$, $c = 71.825$ Å. The structure was solved by molecular replacement with mouse MICAL1 (PDB entry 4txi; Alqassim et al., 2016) and the NMR structure of the CH domain of human MICAL3 (PDB entry 2d88; RIKEN Structural Genomics/Proteomics Initiative, unpublished work) as the initial models. Molecular replacement was performed with Phenix, model building was performed with Coot and final refinement was conducted with REFMAC5 in the CCP4 suite (Liebschner et al., 2019; Emsley et al., 2010; Winn et al., 2011).

2.5. Electron microscopy

The purified sample of human MICAL3FMOCH was diluted with 50 mM Tris pH 8.5, 100 mM NaCl, 1 mM 1,4-dithiothreitol, 1% glycerol to a final concentration of 80 nM. The treated samples (5 μl) were immediately applied onto a carbon-coated grid which had previously been glow-discharged (Harrick Plasma, Ithaca, New York, USA) for 3 min in air. The grids were negatively stained using 1% uranyl acetate. The prepared grids were examined on a Tecnai 10 transmission electron microscope (FEI, USA) equipped with a lanthanum hexaboride (LaB$_6$) cathode operating at 100 kV (the instrumentation used at Kangwon...
2.6. Steady-state kinetic measurements

NADPH consumption was monitored in 96-well microplates at a wavelength of 340 nm using a SpectraMax i3 spectrophotometer (Molecular Devices). All reactions were executed in an F-buffer-based assay mixture. The F-actin was prepared according to the supplier’s instructions. 1 mg of actin from rabbit skeletal muscle (Cytoskeleton Inc.) was reconstituted in 100 μl distilled water. It was diluted to 50 μM in G-buffer (5 mM Tris–HCl, 0.2 mM CaCl₂, 0.2 mM ATP, 1 mM DTT pH 8.0) and incubated for 1 h on ice. Following ultracentrifugation at 100 000 g for 1 h at 4°C, the supernatant that contained G-actin was separated carefully. 10 μl of the supernatant was added instead of the F-actin. All experiments were performed in triplicate, and the reaction was recorded every 10 s for 15 min. Kinetic parameters were calculated by fitting a nonlinear regression to the Michaelis–Menten equation using GraphPad Prism 5.

3. Results

3.1. Overall structure of human MICAL3FMOCH

To gain structural insights into the role of human MICAL3 (hMICAL3) in actin sulfoxidation, recombinant hMICAL3 containing the FMO and CH domains (hMICAL3FMOCH) was expressed and purified for crystallization. The structure of hMICAL3FMOCH was determined at 1.9 Å resolution. hMICAL3 contains multiple domains: FMO, CH, Lin11, Isl-1, Mec-3 (LIM) and C-terminal coiled-coil domains. In particular, the FMO and CH domains of MICAL3 are homologous to the FMO and CH domains of MICAL1 and MICAL2 [Fig. 1(a)] and are known to play a role in methionine sulfoxidation and actin binding, respectively (Hung et al., 2011; Giridharan et al., 2012). The FMO domain of hMICAL3FMOCH has the conserved GXGXXG motif that forms the central part of the consensus hMICAL sequence connecting the first β-strand and α-helix in the βαβ Rossmann fold. hMICAL3 is an oxidoreductase enzyme that depends on FAD and NAD(P) for its catalytic activity (Vanoni et al., 2013). In the structure, Cys97, Val126, Tyr298, Asp398 and Trp405 interact with one FAD molecule. In particular, the indole ring of Trp405 stabilizes the flavin isoalloxazine ring of the FAD via a coaxial stacking interaction, while the other residues interact via hydrogen bonds [Figs. 1(b) and 1(c)]. The crystallographic Rwork and Rfree factors are 20.4% and 22.4%, respectively, and the Ramachandran favored region score is ~98.1% (Table 1). The final model structure consists of the FMO domain (amino acids 10–491) and the CH domain (amino acids 520–627). These two domains are connected by a flexible loop region (amino acids 492–519). Of the 700 amino acids of hMICAL3FMOCH, the 73 amino acids at the C-terminus were

Figure 1 (continued)
Overall structure and characterization of human MICAL3FMOCH. (b) The crystal structure of human MICAL3. The N- and C-termini are labeled N and C, respectively. The FMO domain is shown in yellow and the CH domain is in green. (c) The FAD-binding site. FAD is shown as a stick model and the 2Fo – Fc map for the FAD molecule is contoured at 2σ. The distances between residues and FAD were calculated using PISA. The stick model of human MICAL3 is shown in yellow, white, red and blue, whereas the ribbon model is shown in yellow; the stick and ribbon models of mouse MICAL1 are shown in black.
not observed in the structure. The secondary structure in this region could not be predicted using JPred (Cole et al., 2008), suggesting that this region is flexible.

3.2. CH domain of hMICAL3FMOCH

The CH domain of hMICAL3FMOCH is composed of five $\alpha$-helices and contains a conserved actin-binding motif (522-SKLLGWGQR-530). The hMICAL3FMOCH structure shows that the CH domain interacts with the FMO domain, as is the case in mouse MICAL1 (mMICAL1; Alqassim et al., 2016). In particular, five residues, Lys523, Arg530, Gln531, Tyr620 and Leu627, in the CH domain interact with Lys207, Thr208, Pro210, Glu213 and Glu215 in the FMO domain (Fig. 2). The main chain of Glu213 forms hydrogen bonds to Lys523, and the side chain of Gly213 forms a hydrogen bond and a salt bridge to Tyr620 and Arg530, respectively. In addition, the main chains of Lys207 and Thr208, which correspond to Asn201 and Pro202 of mMICAL1, participate in hydrogen bonding to the side chains of Arg530 and Gln531. Interestingly, the other four binding residues of the FMO domain are not conserved in mMICAL1, except for Glu215 [Fig. 1(a)].

3.3. Interactions between the CH and FMO domains enhance catalytic efficiency

Generally, the type 2 CH domain does not bind to F-actin directly. Instead, it facilitates the binding of F-actin to other parts of the protein (Sun et al., 2006; Gimona & Mital, 1998; Gimona & Winder, 1998). To investigate the effects of the CH domain on MICAL enzyme activity, truncated MICAL proteins with or without the CH domain were produced (hMICAL1FMO, hMICAL1FMOCH, hMICAL3FMO, and hMICAL3FMOCH). It is known that MICAL has NADPH oxidase activity that underlies F-actin disassembly simultaneously with the oxidation of NADPH (Zucchini et al., 2011). Using this property, kinetic parameters for each MICAL form were determined from the initial velocity of the NADPH oxidase reaction, which depends on the NADPH concentration. The initial velocity of the reaction for hMICAL1FMOCH and hMICAL3FMOCH at each NADPH concentration was enhanced by adding F-actin, whereas there was no change in the initial velocity for hMICAL1FMO and hMICAL3FMO when F-actin was added [Figs. 3(a) and 3(b)]. When F-actin was not added, the $k_{cat}/K_m$ value for hMICAL3FMOCH (~2.4794 s$^{-1}$ M$^{-1}$) was similar to that for hMICAL3FMO (~2.0776 s$^{-1}$ M$^{-1}$). However, the $k_{cat}/K_m$ value for hMICAL3FMOCH (~30.9857 s$^{-1}$ M$^{-1}$) increased dramatically on adding F-actin, while the $k_{cat}/K_m$ value for hMICAL3FMO (~2.8793 s$^{-1}$ M$^{-1}$) changed little even after adding F-actin (Table 2). This increase in catalytic efficiency

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<th>Protein</th>
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<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$K_m$ (\mu M)</th>
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Table 2
Steady-state kinetic parameters of various MICALs.

Figure 2
Binding site between the CH domain and the FMO domain in human MICAL3. Residues that participate in the interaction between the FMO and CH domains are labeled. The FMO domain is shown in yellow and the CH domain is shown in green. The binding residues between the FMO domain and the CH domain in human MICAL3 are shown as stick models. The dotted lines indicate the interaction distances between residues.
On adding F-actin when the CH domain is present was also observed in hMICAL1. Nevertheless, the ratio of the increase was much higher in hMICAL3FMOCH (~15 times) compared with hMICAL1FMOCH (~6 times). Consequently, these results reveal that the CH domain of hMICAL3 participates in increasing the F-actin substrate specificity, leading to enhanced catalytic efficiency. Moreover, it appears that the CH domain of hMICAL3 might make a more efficient interaction with the FMO domain for F-actin substrate specificity relative to the CH domain of hMICAL1. We chose two residues, Glu213 in the FMO domain and Arg530 in the CH domain, which were predicted to be key residues associated with the interaction between the FMO and CH domains and mutated them to examine the effect of disrupting the FMO–CH interaction on the catalytic efficiency (hMICAL3FMOCHΔ213,530; E213G and R530G). Notably, the kinetic parameters of hMICAL3FMOCHΔ213,530, including the initial velocity and catalytic efficiency, are more similar to those of hMICAL3FMO than hMICAL3FMOCH [Figs 3(b) and 3(c) and Table 2]. Moreover, F-actin does not increase the catalytic efficiency of hMICAL3FMOCHΔ213,530. Therefore, this result suggests that the FMO–CH interaction in hMICAL3 is required to increase the catalytic efficiency by conferring specific binding to F-actin.

3.4. Structural comparison of CH domains between mouse MICAL1 and human MICAL3

The sequence similarity between the FMO domains of hMICAL3 (residues 1–494) and mMICAL1 (residues 1–489) is ~58%. The FMO domains showed structural similarity, with a root-mean-square (r.m.s) deviation of 0.56 Å when 397 Cα atoms were aligned in PyMOL. Likewise, the CH domains showed structural similarity, with an r.m.s deviation of 0.95 Å, and were considered to be type 2 CH domains (Fig. 4; Janson et al., 2017). However, the relative position of the CH domain of hMICAL3 (hMICAL3CH) in the crystal structure was different from that in mMICAL1. The CH domain can potentially occupy three positions (named positions A, B and C) in the context of an asymmetric unit [Fig. 5(a)]. It was reported that the CH domain of mMICAL1 was located at position B (Alqassim et al., 2016), whereas our structure showed that the CH domain of hMICAL3 was located at position A, where it was rotated approximately 90° from position B [Fig. 5(b)]. There is an invisible region (residues 495–517) between the C-terminus of the FMO domain and the N-terminus of the CH domain in the hMICAL3FMOCH structure, with a distance of ~25.3 Å. Essentially, the calculated distances between the FMO and CH domains are ~25.3 Å for position A, ~48.8 Å for position B and 68.9 Å for position C. Therefore, considering the distance of the invisible region, the CH domain of hMICAL3 fits position A. Furthermore, we performed electron microscopy followed by single-particle analysis to identify the relative position of the CH domain in hMICAL3 (Fig. 6). The visual similarity seen in 3D reconstruction and 2D class averages with corresponding views of raw images.
[Fig. 6(a)] and the fitting of the crystal structure of MICAL3 to the 3D volume of negatively stained MICAL3 molecules supports the idea that the CH domain is located at position A [c.f. the 3D reconstruction in Fig. 6(b) and the models in Fig. 5(a)]. We then built an initial model of the MICAL3\textsubscript{FMOCH–F-actin} interaction [Fig. 6(c)] using position A; the active site seems to be close to the D-loop where the target methionine residues are found.

4. Discussion

MICALs are involved in actin cytoskeleton reorganization through methionine oxidation. However, our understanding of MICALs is limited to genetic and cell biology results, which have been presented in recent papers (Vanoni et al., 2013; Giridharan & Caplan, 2014; Lim et al., 2019). To broaden the scope of these findings, further structural research is required to understand the exact mechanism of how MICALs oxidize methionine. The full-length MICAL protein contains FMO, CH and LIM domains, has a C-terminal domain with unknown function and is highly insoluble, which makes it difficult to obtain a crystal structure. However, its truncated form containing the FMO and CH domains is much more soluble than the full-length form but still retains catalytic activity for F-actin disassembly. Thus, the truncated form of MICAL has been used in various biochemical and biological experiments to gain structural insight into the catalytic mechanism. Of the three mammalian MICALs, only the structure of mouse MICAL1 has been reported and the structure is of a truncated form.
form containing the FMO and CH domains. Interestingly, \textit{mMICAL1\textsubscript{FMOCH}} and \textit{hMICAL3\textsubscript{FMOCH}} are structurally highly similar and share the same catalytic function of depolymerizing F-actin via oxidation of the two conserved methionine residues (Fig. 4). Nevertheless, the crystal structure and electron-microscopy data of \textit{hMICAL3\textsubscript{FMOCH}} show that the spatial arrangement of the FMO and CH domains differs from that in \textit{mMICAL1} (Figs. 5 and 6). In the crystal structure of \textit{hMICAL3\textsubscript{FMOCH}}, three possible positions of the CH domain could arrange in an asymmetric unit (Fig. 5) and one of them matches with the observations from electron microscopy.

These data also suggest that the crystal structure of \textit{mMICAL1} differs from the solution shape from SAXS data (Alqassim \textit{et al.}, 2016).

Both crystal structures clearly show differences, which include the relative location of the CH domain and the length of the invisible region between the FMO and CH domains. The invisible region between the FMO and CH domains is 23 amino acids (495–517) in \textit{hMICAL3} and 18 amino acids (490–507) in \textit{mMICAL1}. Therefore, this could suggest that different binding conformations are adopted by the FMO and CH domains in \textit{hMICAL3} and \textit{mMICAL1}. Although \textit{hMICAL3} and \textit{mMICAL1} have highly conserved FMO and CH domains (Fig. 3), there are several reasons why MICAL3 and MICAL1 may have a different mechanism. Firstly, in the FMO domain of \textit{hMICAL3} the loop is longer than that in \textit{mMICAL1} and \textit{hMICAL1} (Fig. 7). The sequence of this loop is conserved in \textit{hMICAL3} and \textit{hMICAL2}, but is not conserved in MICAL1. However, the sequence of this loop in MICAL1 is conserved in the human and mouse enzymes (Fig. 7). Therefore, MICAL2 and MICAL3 may have a similar binding mode by the type 2 CH domain, but MICAL1 does not. \textit{hMICAL3} and \textit{mMICAL1} are biologically similar in structure, but it is difficult to determine whether they have the same mechanism (Wu \textit{et al.}, 2020).

![Figure 6](image)

Figure 6  
Electron-microscopic analysis of human MICAL3\textsubscript{FMOCH} and a model of MICAL3\textsubscript{FMOCH}–F-actin interaction. (a) Structural comparison taken from 3D analysis: representative surface views of the reconstructed 3D structure (top row) and the corresponding views of 2D class averages (middle row) and raw particles (bottom row). The 10 nm scale bar applies to all of the panels in (a). (b) Superimposition of an equivalent view of the crystal structure (yellow) on the 3D envelope of negatively stained human MICAL3. (c) The initial model was built by manually docking human MICAL3\textsubscript{FMOCH} to F-actin (PDB entry 3luu; Galkin \textit{et al.}, 2010). The CH domain (dark blue cartoon) was oriented first and the FMO domain (purple cartoon) was arranged so that the active site was close to actin. Each actin monomer is represented in a different color (tinted surfaces).

![Figure 7](image)

Figure 7  
Comparison of the FMO domain and CH domain of MICALs from human and mouse. The sequence alignments between the MICALs are shown on the right for the comparison regions shown on the left. The boxes on the left have the same colors as those highlighting the corresponding sequences in Fig. 1. In the top left panel, the loop region of the FMO domain of human MICAL3 is in yellow and red indicates the FMO domain of mouse MICAL1 (PDB entry 4txi). The middle and bottom left panels show the superimposition of the CH domain of human MICAL3 in yellow, human MICAL2 (PDB entry 2e9k; RIKEN Structural Genomics/Proteomics Initiative, unpublished work) in cyan and human MICAL1 (PDB entry 2dk9; Sun \textit{et al.}, 2006) in red.
et al., 2018). In the middle and bottom panels of Fig. 7, the superimposition of hMICAL3, hMICAL2 and mMICAL1 shows that the α-helices of hMICAL2 and hMICAL3 completely superimpose but the α-helix of mMICAL1 is only shifted slightly. This sequence also shows that hMICAL3 and hMICAL2 are completely conserved and mMICAL1 is not conserved at all. Therefore, we suggest that hMICAL3 and hMICAL2 may have a similar mechanism and can be grouped into the same class.

The FMO domain that exhibits monooxygenase activity is localized at the N-terminus of MICAL and is highly conserved among species. The CH domain that is usually found in actin-binding proteins is adjacent to the FMO domain and is also highly conserved. CH domains are classified into three types: types 1, 2 and 3. Whereas type 3 CH domains are mainly found in regulatory proteins associated with muscle contraction and signaling proteins, type 1 and 2 CH domains are usually found in cytoskeletal proteins (Zhou et al., 2011). MICALs have a typical type 2 CH domain. The results of kinetic experiments with hMICAL1 and hMICAL3 reveal that when F-actin is present as a substrate, MICALFMOCH shows a much higher catalytic efficiency than MICALFMO. In the case of MICALFMO, there was no significant change in the activity depending on the presence of F-actin as a substrate. These kinetic data demonstrate that the FMO domain has catalytic activity but that the CH domain must be present for substrate specificity. Moreover, it was shown that the activity of MICALFMO is slightly higher than that of MICALFMOCH without F-actin. These two results suggest that the FMO domain performs the catalytic function regardless of the CH domain but that the CH domain is essential for the F-actin substrate specificity of MICALs. In addition, type 2 CH domains do not possess the ability to bind directly to F-actin, as described previously (Vanoni et al., 2013; Giridharan & Caplan, 2014; Zhou et al., 2011). Therefore, we conclude that the interaction between the FMO and CH domains might generate the substrate specificity, particularly for F-actin.

We also examined the change in catalytic efficiency upon mutating the MICALFMOCH protein by replacing Glu213 and Arg530, two residues that are important for maintaining the interaction between the FMO and CH domains. Upon mutation, the catalytic efficiency is reduced to levels consistent with hMICALFMO. Thus, these findings consistently support the idea that interaction between the FMO and CH domains increases the F-actin substrate specificity and the subsequent catalytic efficiency. Finally, although MICAL has catalytic activity for methionine oxidation, only actin is currently known to be its substrate. The mechanism of sulfoxidation remains disputed and is considered to be owing to direct oxidation by the transfer of a single oxygen molecule or via an indirect oxidation by the production of reactive oxygen species such as hydrogen peroxide. However, it was found that consumption of NADPH without F-actin substrate produced hydrogen peroxide in MICAL1 and MICAL3, but this was not proportional to the activity level of F-actin disassembly (Wu et al., 2018). Consequently, these findings suggest that the CH domain is crucial for conferring F-actin disassembly via methionine oxidation. In this context, the CH domain of MICAL3 interacts with the FMO domain in more efficient ways than the CH domain of MICAL1.


