Membrane Association, Mechanism of Action, and Structure of Arabidopsis Embryonic Factor 1 (FAC1)*§

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Embryonic factor 1 (FAC1) is one of the earliest expressed plant genes and encodes an AMP deaminase (AMPD), which is also an identified herbicide target. This report identifies an N-terminal transmembrane domain in Arabidopsis FAC1, explores subcellular fractionation, and presents a 3.3-Å globular catalytic domain x-ray crystal structure with a bound herbicide-based transition state inhibitor that provides the first glimpse of a complete AMPD active site. FAC1 contains an (α/β)n-barrel characterized by loops in place of strands 5 and 6 that places it in a small subset of the amidohydrolase superfamily with imperfect folds. Unlike tetrameric animal orthologs, FAC1 is a dimer and each subunit contains an exposed Walker A motif that may be involved in the dramatic combined Km (25–80-fold lower) and Vmax (5–6-fold higher) activation by ATP. Normal mode analysis predicts a hinge motion that flattens basic surfaces on each monomer that flank the dimer interface, which suggests a reversible association between the FAC1 globular catalytic domain and intracellular membranes, with N-terminal transmembrane and disordered linker regions serving as the anchor and attachment to the globular catalytic domain, respectively.

Embryonic factor 1 (FAC1)§ was recently identified as one of the earliest expressed plant genes and is essential for the zygote to embryo transition in Arabidopsis thaliana (1). The zygote-lethal phenotype is characterized by developmental arrest at the 8–16-cell stage and mutant embryo shriveling 2–3 days after fertilization. The Arabidopsis FAC1 locus encodes an AMP deaminase (AMPD; EC 3.5.4.6), which is a eukaryotic enzyme that catalyzes the hydrolytic deamination of AMP to IMP. AMPD has also been identified as the intracellular target for their nucleotide derivatives. Taken together, these observations suggest that AMPD is essential throughout the plant life cycle. However, the underlying basis for lethality of a FAC1 null phenotype and for herbicidal toxicity related to the catalytic inhibition of this enzyme is not known.

AMPD catalyzes the initial step in adenosine to guanine ribonucleotide conversion and also in one of the four identified pathways of AMP catabolism that are illustrated in Fig. 1. Each catabolic route differs in the order by which the phosphate, ribose sugar, and 6-amino group are removed from the purine ring structure, then all routes rejoin at the level of hypoxanthine (Hx), which is then further catabolized. Most organisms contain the necessary enzymes for more than one of these pathways. For example, AMP catabolic flow in eukaryotes of the animal kingdom proceeds along pathways 1 and 2. Pathways 3 and 4 are unique to prokaryotes, but these organisms also use pathway 2. Conversely, plants use only pathway 1 because they lack ADA and adenosine (ADEase) (7). Consequently, in plants AMPD is absolutely required for catabolism of AMP to hypoxanthine, which is then oxidized and linearized to form the recyclable ureides, allantoin and allantoic acid.

Although not as well characterized as orthologs in the animal kingdom, endogenous plant AMPD enzymes reportedly exhibit unique physical and regulatory behaviors. Sequence analysis of available cDNA for the single A. thaliana gene (At2g38280) identifies motifs that could play structural and regulatory roles in plant-specific properties of AMPD. For example, a putative N-terminal helical transmembrane domain (residues 6–31) may be responsible for the particulate distribution of plant enzymes, as reported in pea seeds (3, 10), spinach leaves (11), and artichoke tubers (7, 12). This behavior results in poor yields and impedes further purification efforts. In addition, a putative ATP/GTP binding motif (Walker A ((A,G)VXGK(S,T)), residues 289–296) may be involved in a dramatic Km and Vmax effect of ATP, as observed with the Catharanthus roseus enzyme (13).

AMPD is one of more than 1000 documented enzymes in the amidohydrolase superfamily (14). The most recognizable feature across the 16 unique members for which high-resolution x-ray crystal structures are available, including the functionally related ADA (15, 16), is a...
mononuclear or binuclear metal center embedded within an (α/β)_8-barrel structural fold. It has long been proposed that the functional conformation of AMPD would resemble that of ADA due to similarity of the catalyzed reactions and the potent inhibition of both enzymes by deoxycoformycin derivatives (6). Although there is very little overall sequence homology between these two enzymes, many of the essential catalytic residues in the active site of ADA (15, 16) are conserved in AMPD, including a signature motif, SLIS/NNTDDP (17).

This study explores the relationship between a putative N-terminal transmembrane domain in the A. thaliana FAC1 polypeptide and the particulate behavior of this plant enzyme. Physical and catalytic properties of FAC1 are also measured using purified soluble N-truncated enzymes. Polyclonal antiserum raised against one of these purified enzymes is used to examine the subcellular fractionation and the membrane extraction of FAC1 in Arabidopsis T87 protoplasts. Finally, because of its central role in nucleotide metabolism and the lack of sequence homology to any structure in the Protein Data Bank, the Center for Eukaryotic Structural Genomics undertook a structure determination. The x-ray crystal structure of the globular catalytic domain of FAC1 is presented with bound herbicide-based transition state inhibitors to reveal connections of function with structure.

**EXPERIMENTAL PROCEDURES**

Expression and Purification of FAC1 Recombinant Enzymes—Recombinant plasmids containing wild type and N-truncated (ΔL31M and ΔI139M) FAC1 cDNA sequence (see supplementary Materials and Methods) were co-transfected into Spodoptera frugiperda (SB9) cells together with a modified baculoviral genome (BaculoGold; BD Pharmingen) and the recombinant viral plaques were purified and amplified. Recombinant virus was used to infect 12 confluent T-185 flasks of Sf9 cells and recombinant enzymes were purified by phosphocellulose chromatography using a previously described protocol (18). Unless otherwise stated, leupeptin was included in all extraction and storage buffers to minimize N-terminal proteolysis, as previously described (19).

Subcellular Fractionation Analysis of FAC1 Distribution in Arabidopsis T87 Protoplasts—Subcellular fractionation of Arabidopsis suspension cultured cells was performed as described (20) in membrane isolation buffer (MIB: 20 mM HEPES-KOH, pH 7.0, 50 mM KOAc, 1 mM Mg(OAc)₂, 250 mM sorbitol) supplemented with 1 mM dithiothreitol (DTT), and a protease inhibitor mixture containing 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml pepstatin A, 1 μg/ml chymostatin, 1 mM p-aminobenzamidin, 1 mM ε-aminocaproic acid, 5 μg/ml aprotinin, 1 μg/ml leupeptin, 10 μg/ml E64d. The subcellular distribution and activity of FAC1 were analyzed by immunoblotting and enzyme assay, which included 1 mM ATP to achieve maximal enzyme activity. To characterize the membrane association of FAC1, 223 μg of protein in the P150 fraction was diluted (10-fold) into MIB containing 1 mM DTT (MIBDTT), or MIBDTT supplemented with either 2.5 mM NaCl, 100 mM Na₂CO₃, pH 11.5, or Triton X-100 (1% (v/v)). Samples were incubated for 30 min on ice followed by centrifugation (Beckman TLA100.1, 90,000 rpm, 30 min, 4°C). Pellet fractions were washed with MIBDTT and 20 μg of protein equivalents of supernatant and pellet fractions were analyzed by immunoblotting using antisera specific for FAC1 (see supplementary Materials and Methods), cytosolic (PUX1) (21), integral membrane (AtSEC12) (22), and peripheral membrane (AtCDC48) (20) proteins. Prior to immunoblotting, the nitrocellulose membrane was analyzed by Ponceau S staining to confirm protein recovery and equal loading.

Crystallization and Structure Determination of ΔI139M FAC1 Recombinant Enzyme in Complex with a Transition State Inhibitor—Crystals of ΔI139M FAC1 recombinant enzyme in complex with a transition state analogue inhibitor, coformycin 5'-phosphate (Fig. 6C), were obtained by hanging-drop vapor diffusion at 22 °C. The precipitant solution contained 0.4 M monoaammonium dihydrogen phosphate, 0.1 M trisodium citrate, pH 5.6, and 10% (v/v) ethanol, as previously described (23). Native and a mercury derivative data set (thermosal), respectively, were collected at synchrotron beamlines 22-ID and 19-ID at the Advanced Photon Source of the Argonne National Laboratory as previously described (23). The data sets of diffraction images were integrated and scaled using the HKL2000 suite (24). ΔI139M FAC1 structure was phased using the molecular replacement method with the rabbit AMPD1 structure (25) as a phasing model by MolRep (26) in the CCP4ii suite (27). The model was improved using alternate cycles of manual building in Xfit (28) and refinement in REFMAC5 (29), and the final structure was refined with the Crystallography & NMR System (CNS) (30).

Normal Mode Analysis (NMA) of ΔI139M FAC1 Dimer—The dynamical domains in the ΔI139M FAC1 dimer were determined using NMA (31) in the Molecular Modeling ToolKit (32). A deformation measure was calculated for the carbon α (Ca) atom in each residue followed by normal mode calculations. The two lowest frequency non-trivial normal modes were combined and the results were scaled with a heuristic scaling factor.

**AMPD Enzyme Assay**—AMPD activity was measured in 100-μl reactions containing 25 mM imidazole, pH 7.0, 150 mM ammonium sulfate, 20 μg of bovine serum albumin, and 20 mM ATP. Substrate and product were resolved and quantified by anion-exchange high pressure liquid chromatography as previously described (33, 34). Kinetic studies were conducted under the assay conditions described above using ~40 milliunits of enzyme per assay, variable substrate concentrations (0.017–106 mM AMP), and initial velocity conditions (product not exceeding 15% of substrate). Kinetic parameters were calculated by fitting data to the following equation: log v = log[(VA/(K + A)], where A was AMP concentration and V was the rate. A log fit was called for because data measurement errors were proportional to the velocities. Residual (difference between experimental and calculated V) did not
exceed 2.6 σ (the 99 percentile criterion for throwing out a point and refitting) for any fit.

Coordinates—The coordinates and diffraction data of the ΔI139M FAC1 structure in complex with coformycin 5′-phosphate have been deposited in the Protein Data Bank (accession code 2A3L).

RESULTS

Expression and Purification of FAC1 Recombinant Enzymes—FAC1 recombinant enzymes were expressed in S9 insect cells using baculoviral technology. Expression of the full-sized FAC1 cDNA produces a robust activity that partitions predominantly into the particulate fraction of insect cells, as evidenced by the low recovery from a freshly prepared sonicate following a 10,000 g spin (see supplementary Table 1). However, there is a time-dependent increase in recovery when the sonicate is stored at 4 °C in the absence of protease inhibitors prior to centrifugation. SDS-PAGE analysis of the peak fraction eluting from a phosphocellulose column following adsorption of crude extract derived from a sonicate stored at 4 °C for 93 h reveals a series of bands between 70 and 95 kDa (see supplementary Fig. 1). These combined data suggest that proteolytic events occur in the sonicate over time at 4 °C and remove a region of the 95-kDa FAC1 polypeptide that is responsible for the particulate distribution of this enzyme. Furthermore, this occurs without any substantial loss of catalytic activity, as evidenced by similar total sonicate activities over this period of time (see supplementary Table 1).

To test this hypothesis, two N-truncated enzymes, ΔL31M and ΔI139M, were constructed and expressed. Both enzymes are soluble and have robust activities (see supplementary Table 1), and phosphocellulose chromatography purification in the presence of protease inhibitor (leupeptin) followed by SDS-PAGE fractionation yields single bands of approximate 92 and 80 kDa (see supplementary Fig. 1), the predicted masses of the ΔL31M and ΔI139M polypeptides, respectively. Notably, the specific activities of these purified preparations are 3 orders of magnitude higher than those previously reported for endogenous plant AMPD enzymes (120–458 milliunits/mg of protein) (3, 11–13). The combined results demonstrate that up to 31 N-terminal amino acids in the FAC1 polypeptide are responsible for partitioning of the wild type enzyme into the particulate fraction.

Subcellular Fractionation Analysis of FAC1 Distribution in Arabidopsis T87 Cells—Rabbit polyclonal antiserum was raised against purified ΔI139M antigen and this reagent was used to examine FAC1 distribution and membrane extraction in T87 cells. AMPD enzyme activity and immunoreactive polypeptide are located exclusively in the microsomal membrane fraction (P150) of these cells (Fig. 2). Furthermore, high salt (2.5 M NaCl), high pH (Na2CO3, pH 11.5), and anionic detergent (Triton X-100) all remove a portion of FAC1 from the P150 fraction, but the majority of immunoreactive polypeptide is not extracted into the soluble fraction under any of these conditions. Conversely, an integral membrane protein with a single transmembrane domain, AtSEC12, is extracted by anionic detergent, but not by either high salt or pH. In addition, a peripheral membrane protein, AtCDC48, is extracted by high pH and partially extracted by high salt.

Effect of ATP on the Kinetic Properties of FAC1 Recombinant Enzymes—As reported for isolated pea seed AMPD (10), the ΔL31M and ΔI139M FAC1 recombinant enzymes prefer sulfate anion for maximal activity (data not shown), rather than chloride, which is typically used to study
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The catalytic behaviors of animal orthologs. Therefore, sulfate anions were included in the assay buffer for the kinetic analysis of these two recombinant enzymes. In the absence of ATP, $K_m$ and $V_{\text{max}}$ values for the ΔL31M enzyme are 6.7 ± 0.8 mM and 68 ± 6 units/mg of protein, respectively, and 12 ± 3 mM and 17 ± 3 units/mg of protein, respectively, for the ΔL139M enzyme. $V_o$ versus [S] plots presented in Fig. 3 show that 1 mM ATP exerts a combined $K_m$ and $V_{\text{max}}$ effect on both enzymes. $K_m$ and $V_{\text{max}}$ values for the ΔL31M enzyme are 0.26 ± 0.03 mM and 375 ± 25 units/mg of protein, respectively, and 0.15 ± 0.02 mM and 113 ± 7 units/mg of protein, respectively, for the ΔL139M enzyme.

**Overall Structure of ΔL139M FAC1 in Complex with Coformycin 5'-Phosphate**—The ΔL139M FAC1 recombinant enzyme was crystallized with bound coformycin 5'-phosphate. The crystals belong to space group P6$_2$2$_1$P6$_2$2$_1$, and comprises 616 residues (212–273 and 286–839), one coformycin 5'-phosphate, and one phosphate ion. Statistics for data collection and refinement are summarized in Table 1. An anomalous difference Fourier map generated with the mercury data set revealed six collection and refinement are summarized in Table 1. An anomalous difference Fourier map generated with the mercury data set revealed six

| Table 1 Data collection and refinement statistics |
|-----------------------------------------------|
| Data collection | ΔL139M FAC1 | Mercury derivative |
| Complex with coformycin 5'-phosphate | P6$_2$2 | P6$_2$2 |
| Space group | P6$_2$2 | P6$_2$2 |
| Cell dimensions | a, b, c (Å) | 131.3, 131.3, 208.3 | 132.6, 132.6, 207.6 |
| α, β, γ (°) | 90, 90, 120 | 90, 90, 120 |
| Resolution (Å) | 50.00–3.34 (3.42–3.34) | 50.00–4.05 (4.12–4.05) |
| R$_{free}$ | 0.06 (0.49) | 0.12 (0.36) |
| I/σ$_i$ | 30.6 (4.1) | 26.5 (10.3) |
| Completeness (%) | 99.5 (99.6) | 99.9 (100) |
| Redundancy | 6.1 (5.7) | 17.0 (17.8) |
| Refinement | Resolution (Å) | 50.00–3.34 |
| No. reflections | 15,038 |
| R$_{free}$/R$_{merge}$ | 0.237/0.323 |
| No. atoms | |
| Protein | 5050 |
| Ligated/ion | 30 |
| Water | 24 |
| B-factors | |
| Protein | 73.47 |
| Ligated/ion | 77.27 |
| Water | 75.90 |
| Root mean square deviations | |
| Bond lengths (Å) | 0.016 |
| Bond angles (°) | 2.40 |

*Values in parentheses are for the highest resolution shell.

$R_{merge} = \frac{\sum_i |I_i(h)−\langle I(h)\rangle|^2}{\sum_i |I_i(h)|^2}$, where $I_i(h)$ is the intensity of an individual measurement of the reflection and $\langle I(h)\rangle$ is the mean intensity of the reflection. $R_{free} = \langle F_{\text{calc}}−|F_{\text{obs}}|\rangle/\langle F_{\text{calc}}\rangle$, where $F_{\text{calc}}$ and $F_{\text{obs}}$ are the observed and calculated structure-factor amplitudes, respectively.

$R_{merge}$ was calculated as $R_{free}$ using 5.0% of the randomly selected unique reflections that were omitted from structure refinement.

The active site of FAC1 with bound coformycin 5’-phosphate is positioned on the C-terminal side of the imperfect (α/β)$_2$ barrel, surrounded by multiple helices and loops. The catalytic zinc ion is coordinated to the coformycin 5’-phosphate, an aspartic acid (Asp$^{736}$) and three histidine (His$^{939}$), His$^{939}$, and His$^{939}$ residues (Fig. 6A). There are 7 identified variations of the metal ligand centers in the amidohydrolase superfamily (14), and this configuration is referred to as subtype III. A fourth histidine (His$^{939}$) not ligated to the zinc resides on loop 6 and is positioned to function in catalysis as a general base during proton abstraction from the water molecule that is complexed with this ion. Alternatively, a glutamate residue (Glu$^{662}$) is similarly positioned and could perform this catalytic role. Residues Phe$^{663}$ and Tyr$^{667}$ are important in positioning the inhibitor in the FAC1 active site by causing the ribose ring to assume a different orientation compared with the coformycin base (Fig. 6B).

The phosphate group of the inhibitor is located in a polar environment immediately to the left of the catalytic zinc and spans residues 289–296
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FIGURE 4. Overall structure of FAC1. A, stereo view of the monomeric structure. The active site of FAC1 with catalytic zinc and coformycin 5′-phosphate is located at the C-terminal side of the incomplete TIM barrel. The catalytic zinc is represented as a blue sphere, the coformycin 5′-phosphate and the Walker A motif are shown as orange stick models, and the phosphate ion is depicted as an orange sphere. Secondary structures of FAC1 are colored differently: α-helices and loops in green and β-sheets in purple. The drawing (lower right) illustrates the point of view (indicated by a black arrow), B, dimeric structure. One monomer is colored in green, and the other is in magenta. Exposed Walker A motifs (Ala289–Ser296) are represented by the red surfaces and are located 28 Å from the respective catalytic zinc ions. Phosphate ions are shown as orange, red, and gray spheres and are located at the N-terminal side of the incomplete TIM barrels and are located 30 Å from the respective catalytic zinc ions. Relative orientations of the incomplete TIM barrel in each FAC1 monomer of the dimeric structure are indicated. Figures were prepared using PyMol program.

(AHYPQGKS) that comprise part of a loop structure exposed to solvent in both subunits of the physiological dimer. The phosphate ion is located at the N-terminal side of the imperfect TIM barrel 30 Å from the catalytic zinc and is surrounded by several basic residues (Arg380, Arg386, and Lys506, not shown).

NMA of ∆I139M FAC1 Dimer—NMA of the ∆I139M FAC1 dimer shows that it likely moves with an opening and twisting hinge motion (Fig. 5B) that would make the basic surface of each monomer flat with respect to each other (compare Fig. 5, C and D; see supplementary Movie 1). The distance between the α-carbon atoms of residue Arg351, a basic residue located in the middle of helix 3, would increase from 50 Å in the crystal structure to 71 Å in the flattened form. In addition, the distance between the α-carbon atoms of residue Ala289 in the Walker A motif would decrease from 56 to 41 Å. Finally, the distance between the two catalytic zinc ions would increase from 40 to 45 Å, which slightly widens the distance between the two active sites. These structural rearrangements could be involved in allosteric switching.

DISCUSSION

The FAC1 gene encodes AMPD, an enzyme that catalyzes a pivotal reaction in purine nucleotide interconversion and catabolic pathways, and which has been extensively studied in many species of the animal kingdom. Conversely, AMPD has not been well characterized in any plant species. This study has taken advantage of molecular resources available for Arabidopsis thaliana to advance our understanding of FAC1 and has revealed distinguishing features of the encoded enzyme. For example, several algorithms (TMAP, DAS, TMpred, and SOSUI) predict a 26-residue helical transmembrane domain in the N terminus of the FAC1 polypeptide (residues 6–31), which is conserved in the rice (Oryza sativa) sequence (53% identity across 26 residues, shaded in Fig. 7), and subcellular fractionation reveals partitioning of AMPD enzyme activity and immunoreactive polypeptide into the microsomal membrane fraction of Arabidopsis T87 protoplasts. However, whereas FAC1 clearly associates with membranes, the nature of this interaction is less obvious, as evidenced by partial solubility of the immunoreactive polypeptide in the presence of high salt or high pH. Typically, peripheral membrane proteins are completely solubilized at high pH, whereas integral membrane proteins are completely solubilized by detergents. Yeast Tim14 (36, 37) and Rieske Fe/S protein (37) are notable exceptions because they are extracted from the inner mitochondrial membrane at high pH despite spanning the lipid bilayer with a single transmembrane domain. The previous observation that Jerusalem artichoke mitochondria are enriched in AMPD activity (38) suggests that FAC1 may belong to this class of proteins.

The membrane association of FAC1 also explains why previous studies in pea seeds (3, 10), spinach leaves (11), and artichoke tubers (7, 12) were limited by the particulate nature of these enzymes, which resulted in poor purification yields that impeded further characterization. Baculoviral expression of FAC1 wild type cDNA also produces an enzyme that partitions (>95%) into the particulate fraction of an insect cell sonicate. However, storing the sonicate at 4 °C prior to centrifugation results in a time-dependent increase of soluble enzyme. AMPD polypeptides in the animal kingdom are sensitive to N-terminal proteolysis (39) and a similar phenomenon may remove the membrane...
anchoring sequence responsible for the particulate behavior of the FAC1 enzyme. This hypothesis was confirmed by showing that deletion of 5'-cDNA sequence encoding 30 N-terminal amino acids results in a soluble enzyme.

Because full-length FAC1 is difficult to characterize, purified N-truncated recombinant enzymes were examined and kinetic data indicate a strong dependence on ATP. \( K_m \) values for FAC1 recombinant enzymes are 25–80-fold lower in the presence of this nucleotide effector, and are similar to those previously reported for partially purified AMPD enzymes from other plant species (10–13). ATP also produces a 5–6-fold increase in the \( V_{max} \) of FAC1, which is similar to that observed with the \( C. \) roseus enzyme (13) but not with any AMPD ortholog in the animal kingdom. The remarkable ATP activation of FAC1 by this combined \( K_m \) and \( V_{max} \) effect may involve a Walker A motif \( ((A,G)X_4GK(S,T)) \), also not found in any animal AMPD ortholog. This potential ATP/GTP binding site spans residues 289–296 (AHYPQGKS) in the FAC1 polypeptide and the x-ray crystal structure of the \( \Delta \)1139M enzyme shows this sequence as part of a loop structure exposed to solvent in both subunits of the physiological dimer (Fig. 4B), where it appears positioned to bind a purine nucleoside triphosphate. The crystallization conditions included ammonium phosphate, which also resulted in the identification of a bound phosphate ion in a basic environment at the N-terminal side of the imperfect TIM barrel in the x-ray crystal structure. This serendipitous outcome is notable because phosphate is an identified allosteric inhibitor of plant AMPD enzymes (10–12).

The x-ray crystal structure also reveals several other unusual features
of the globular catalytic domain of FAC1. For example, unlike the TIM barrel found in most other members of the amidohydrolase superfamily for which there is available high-resolution structure (14), the (α/β)8-fold is imperfect in FAC1. Six of the eight β-strands can be recognized, but the 5th and 6th are replaced by somewhat irregular loop structures. Notably, this variation on the TIM barrel is also found in the x-ray crystal structure of the rabbit AMPD1 enzyme (25). Occasionally there is a deletion or insertion in a strand that causes a single residue interruption of the secondary structure in other amidohydrolase enzymes (14). However, there is no homologous structure in the Protein Data Bank in terms of amino acid sequence, although DALI (40) and VAST (41, 42) searches show weak overall structural similarity with other amidohydrolase enzymes, including ADA. Surprisingly this functionally related enzyme is no more similar than several other members of this superfamily (phosphotriesterase Protein Data Bank code 1PSC; isoaspartyl dipeptidase code 1PO9; and D-hydantoinase code 1YNY).

In addition, FAC1 assembles as a physiological dimer, whereas gel filtration or sedimentation velocity ultracentrifugation combined with SDS-PAGE (19, 43–48), and perhaps x-ray crystallography (25), have shown that AMPD enzymes of the animal kingdom are tetramers. The crystal structure of rabbit AMPD1 reveals two subunits packed around a non-crystallographic dyad axis forming the asymmetric unit. This
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**FIGURE 8.** Paddleball model for the complex formed between FAC1 dimer and an intracellular membrane. The transmembrane domain (residues 6–31) and disordered linker domain (residues 32–211) of both subunits tether the dimeric globular catalytic domain (residues 212–839) to the lipid bilayer. The basic residue-rich surface spanning the dimer interface can become quite flat in the region of positive charge and facilitate interactions with negative patches on the surface of the membrane. Dimer orientations are identical to those in Fig. 5, C (left) and D (right).

dimer is packed around the crystallographic 2-fold axis to give the physiologically observed tetramer. Notably, the FAC1 dimer has a larger contact area (251±Å² or 9.5% of total monomer area) than any two rabbit AMPD1 subunit contacts. Thus, the quaternary structures of plant and animal AMPD are quite different.

Finally, the FAC1 dimer has a flat basic residue-rich surface that spans one side of the dimer. Conversely, positively charged residues are fewer and spread more evenly over the surface of the rabbit AMPD1 structure. NMA of the FAC1 dimer reveals a potential for greater flattening of this positively charged surface, which would enhance the potential for an electrostatic interaction with a phospholipid bilayer.

The final crystallographic model of the Δ1139M FAC1 enzyme comprises only 616 of the 701 amino acids in this polypeptide (residues 212–273 and 286–839). Whereas 13 of the 85 unstructured amino acids are part of a loop that forms the Walker A motif, the remainder are located in the N terminus of this truncated enzyme. Regarding these latter residues, several computer-based prediction servers (ROBETTA, GLOBPLOT, FoldIndex, and DRIPPRED) indicate that the entire stretch of sequence (residues 32–211) between the N-terminal helical transmembrane domain and the globular catalytic domain is largely unstructured. This extended linker domain could be responsible for the faster migration of the ΔL31M enzyme on a gel filtration column relative to that expected for this native dimeric enzyme (see supplementary Fig. 2A). Disordered N-terminal regions have also been proposed for the three tetrameric human AMPD2 spliceforms, which run 30–70% larger than expected on this same gel filtration column (19).

An alignment of available AMPD predicted primary amino acid sequences suggests that the unique tertiary and quaternary structures of FAC1 are likely conserved throughout the plant kingdom (Fig. 7). For example, the rice full-length sequence is 74% identical to the FAC1 polypeptide, including the N-terminal transmembrane domain. In addition, across 642 C-terminal residues that include the entire globular region of the Δ1139M structure, the FAC1 polypeptide exhibits 82% identity with the corn and barley partial sequences (data not shown). Furthermore, nine of the 10 exposed basic residues on the flat surface of each subunit are conserved in these four plant sequences. Conversely, FAC1 aligns with shorter stretches of C-terminal sequence in mammalian AMPD polypeptides and at lower amino acid identities (51–57% over 555–593 C-terminal residues). In addition, mammalian AMPD polypeptides consistently retain only five of the 10 positive charges that comprise the flat surface of the FAC1 subunit structure. However, plant and animal sequences both exhibit a strict conservation of critical residues that coordinate the catalytic zinc, interact with the bound transition state inhibitor, and bind to the allosteric inhibitor phosphate ion in the FAC1 structure (Fig. 7). These include reactive groups that are central to the catalytic mechanism of ADA (15, 16), suggesting that the two enzymes use similar strategies to facilitate the hydrolytic elimination of ammonia at position 6 of the adenine ring.

The active site of FAC1 also offers structural insight related to other previously unresolved issues of AMPD catalytic function. For example, a D598N substitution in the FAC1 enzyme (FAC1–1) reportedly has a milder phenotype than a complete knock-out caused by T-DNA insertion into exon 10 of the gene (FAC1–2) (1). Asp598 is located on β-strand 4 of the imperfect TIM barrel, where it appears to stabilize the N-δ of His659 located on the axial position of the catalytic zinc (Fig. 6A). The distances between the two oxygens of Asp598 and the N-δ of His659 are 3.1 Å, suggesting that Asp598 stabilizes His659. Furthermore, the electron density around one of the oxygens of Asp598 appears in a position to hydrogen bond with Arg517 (see supplementary Fig. 4), which would limit the direction of the other oxygen of Asp598 toward His659. The Asp to Asn substitution at residue 598 replaces one of the oxygens with a nitrogen, which would destabilize the proton bound to the N-δ of His659. Consequently, this could affect the electrostatic configuration of the Zn²⁺ and perturb, but not eliminate, catalytic activity, thus resulting in a less severe phenotype.

Another unresolved issue relates to the well established observation that 2′-deoxy-AMP (dAMP) is not a good substrate for either plant or animal AMPD enzymes (2–16% rate of deamination relative to AMP) (11–13, 48). This can be explained by an apparent interaction between Tyr467 and the 2′-OH of coformycin 5′-phosphate that contributes to the different orientations of the ribose sugar and nitrogen base moieties of this transition state inhibitor in the active site of FAC1 (Fig. 6B). Conversely, ADA can efficiently deaminate dAMP because the 2′-OH of adenosine is not essential for substrate binding (15, 16). This ribonucleotide substrate preference of AMPD has a significant clinical consequence because it promotes deoxyadenosine nucleotide accumulation that produces a severe combined immunodeficiency in an inherited ADA deficiency in man (49).

The combined structural features of FAC1 in conjunction with sequence conservation across the plant kingdom point toward a mechanism of membrane anchoring involving both insertion of the two N-terminal transmembrane domains and electrostatic interactions along the charged, flat surface of the physiological dimer (Fig. 8). The proposed configuration between FAC1 and a lipid bilayer is reminiscent of a “paddleball,” with the globular catalytic domain representing the “ball” and the transmembrane domains (residues 6–31) and disordered linker regions (residues 32–211) comprising the anchors and flexible connectors, respectively. The dynamic aspect of this model predicts a reversible association between the flat surfaces flanking the dimer interface and an intracellular membrane (“paddle”).
Acknowledgments—We acknowledge Dr. Eduard Bitto for many contributions to the solution of the FAC1 structure and Dr. W. Wallace Cleland for help in analyzing the kinetic data. We acknowledge the Structural Biology Center (SBC) at Argonne National Laboratory for use of beamline 19-ID at the Advanced Photon Source, and SBC beamline scientists Randy Alkire, Norma E. C. Duke, and Stephen L. Ginell for assistance during data collection. We also acknowledge the Southeast Regional Collaborative Access Team (SER-CAT) for use of beamline 22-ID (or BM) at the Advanced Photon Source, Argonne National Laboratory.

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