BIOCHEMISTRY

# Remote Enzyme Microsurgery

Protein structures reveal a surprising mechanism for construction of a complex enzyme cofactor from standard amino acids.

J. Martin Bollinger Jr. and Megan L. Matthews

Enzymes achieve astounding rate enhancements of even difficult reactions. They often covalently modify their substrates by their own functional groups, a tactic that enables them to access mechanistic pathways that would not be feasible in solution. The standard amino acid building blocks of enzymes are replete with nucleophiles to use in this "covalent catalysis," but they are essentially devoid of useful electrophiles. An enzyme can bind an exogenous cofactor such as pyridoxal 5′-phosphate in its active site to correct this deficiency. An elegant alternative is the in situ construction of an electrophile, subsequent to protein synthesis, from amino acids in the active site (*1*). On page 1392 of this issue, Jensen *et al*. reveal how a particularly intricate example of this enzyme microsurgery is accomplished (see the figure) (*2*).

In a surprising number of these active-site surgeries, the enzyme is both patient and doctor, directing the construction of its own cofactor. Less commonly, the patient relies on a surgical specialist: an accessory enzyme that catalyzes cofactor construction. The extensive surgery needed to activate methylamine dehydrogenase (MADH) is one such case. Prior to Jensen *et al*.'s study, genetic and biochemical studies had shown that the two-heme protein MauG (*3*) operates on the inactive pre-MADH to install an unusual tryptophan tryptophyl quinone (TTQ) cofactor in the enzyme's β subunit (see the figure) (*4*, *5*). The surgery involves three steps: cross-linking of tryptophan (Trp) and 7-hydroxytryptophan (7-OH-Trp) residues in preMADH; hydroxylation of the 7-OH-Trp at position 6; and dehydrogenation of the cross-linked unit to a quinone. Each step is a two-electron oxidation. MauG can use hydrogen peroxide ($H_2O_2$) or dioxygen ($O_2$) as the oxidizing cosubstrate (*6*).

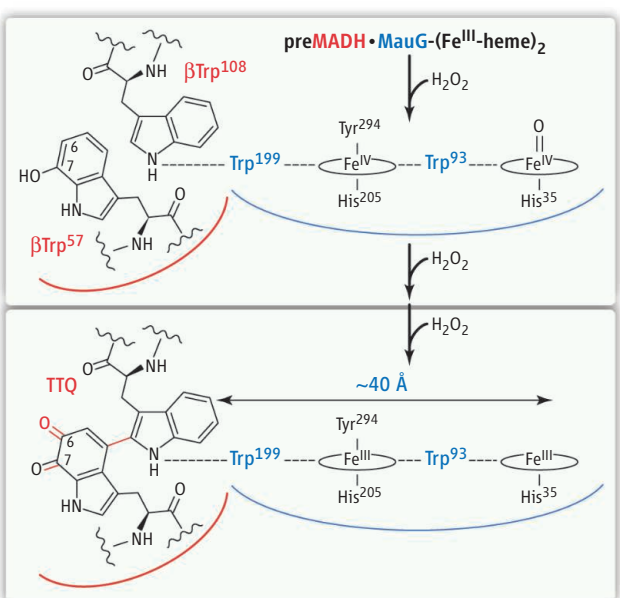
When a surgeon-enzyme such as MauG catalyzes cofactor construction, site access becomes important, because the amino acids to be cut and sutured may be buried in the patient's interior. Protein-protein interactions can drive conformational changes that permit direct access. Before the structural studies of Jensen *et al*., one might have surmised that MauG would perform such an "open" procedure on preMADH. MauG can form a unique intermediate containing one Fe(IV)-oxo (ferryl) site and one non-ferryl Fe(IV); this Fe(IV)/Fe(IV)=O complex oxidizes MADH in the synthesis of TTQ (*7*). Ferryl hemes in other enzymes are known to effect both hydroxylations and radical couplings on aromatic substrates. One might thus have anticipated that engagement of preMADH and MauG would permit the ferryl heme in MauG to directly access the Trp residues in MADH.

Jensen *et al*.'s structures of the (pre) MADH•MauG complexes defy these expectations. Binding of MauG positions its ferryl-forming heme away from the MADH interface, ~40 Å from the target Trp and 7-OH-Trp residues! MauG bound to preMADH in this way can complete TTQ construction upon exposure of the crystals to $H_2O_2$, and the resultant structural changes are essentially localized to the cofactor region. The structures thus imply that TTQ construction is more akin to keyhole surgery, with MauG extracting electrons from MADH sequentially from a long distance, likely generating radical intermediates, until cofactor synthesis is complete.

The structures shed light on several prior mechanistic observations, including the stability of the unique Fe(IV)/Fe(IV)=O intermediate in MauG. On the basis of its Mössbauer spectra, one of the Fe(IV)-hemes had been assigned as having two axial ligands from the protein (*8*), a previously unknown complex. The structures identify the ligands as a histidine and tyrosine, an unprecedented combination for a c-type heme. Bacterial two-heme cytochrome c peroxidases—which are similar to MauG in overall structure, have nearly identical two-heme cofactor regions, and catalyze similar reactions involving $H_2O_2$, but do not form the Fe(IV)/Fe(IV)=O intermediate—have a histidine or methionine residue at the position of the MauG tyrosine (*9*). Thus, the tyrosine ligand to the central heme may be a crucial surgical instrument of MauG. Substitution of this tyrosine with other residues could be used to test this idea.

The structures also offer new mechanistic insights and suggest approaches for further dissection of the surgical mechanism. Having ruled out direct access of MauG to the MADH Trp residues, the authors envisage the sequential extraction of six electrons by the Fe(IV) hemes, most likely in three rounds of the MauG reaction cycle. The hemes are ~15 Å to ~35 Å from the two Trps—a long distance for the electrons to travel. The surgeon uses the enzyme equivalent of a scope, positioning at its MADH interface a Trp residue that most likely relays electrons from the incipient TTQ to its closer, six-coordinate Fe(IV)-heme and a second Trp almost centrally between the two hemes that probably relays electrons between them (*10*). Variants in which the electron-relay Trp residues are substituted by other residues might provide useful probes of the electron-transfer and TTQ-assembly mechanisms.

Jensen *et al*.'s study leaves at least two key questions unan-




**Schematic representation of the MADH•MauG complex and synthesis of the TTQ cofactor therein.** Bonds of the cofactor installed by MauG are shown in red and residues that are part of the surgeon's "scope" in blue.

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swered. First, it is unclear how the C7 hydroxyl group already present on $Trp^{57}$ in preMADH (*11*, *12*) is incorporated. The gene encoding the β subunit specifies Trp at this position, and no surgeon specializing in the initial 7-hydroxylation of $Trp^{57}$ has been identified (*6*).

Second, although the structures provide crucial insight into the fundamental nature of the individual oxidation steps [long-distance electron transfers with Trp relays (*10*)], they do not reveal the identities of partially assembled intermediates and thus do not resolve the full reaction sequence. Elucidation of this sequence remains a daunting challenge, especially because the oxidation steps are apparently processive (*2*). Variant proteins designed with the aid of these structures may permit strategic disruption of the process and accumulation of intermediate forms for kinetic and structural characterization.

Is the remote nature of the posttranslational active-site surgery by MauG on preMADH unique to this system? The answer appears to be "no." A recent paper by Cotruvo and Stubbe examining the class Ib ribonucleotide reductase from *Escherichia coli* implies that the protein NrdI acts as a remote enzyme-surgeon in this system (*13*). As the Jensen *et al*. study so beautifully illustrates, structures of surgeon-patient complexes should reveal important details of this and other remote enzyme surgeries.

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PLANETARY SCIENCE

# Revealing Titan's Interior

Gravity field measurements by the Cassini spacecraft suggest that Titan's interior was too cold for the primordial mixture of ice and rock to melt and fully separate.

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The interior structure and composition of solar system bodies are key to understanding their origin and evolution. Saturn's largest icy moon, Titan, and the jovian moons, Ganymede and Callisto, are of similar size, mean density, and primordial ice-rock fraction from which the satellites formed. Titan is distinct due to its dense nitrogen atmosphere, with methane as the next most abundant constituent, which precludes direct observations of the surface. Before the arrival of the Cassini-Huygens spacecraft to study the Saturn system in 2004, little was known about the nature of Titan's interior—information as to its origin, evolution, and the rate at which it degasses was limited. On page 1367 of this issue, Iess *et al*. (*1*) report evidence based on the analysis of its gravitational field that the interior was much colder than previously thought, and thereby impeded substantial melting and subsequent separation of the primordial ice-rock mixture.

The Cassini-Huygens mission has revealed a remarkable diversity of Titan's icy landscapes, such as branching riverbeds, liquid methane and/or ethane pooled in polar lakes, and wind-blown equatorial dune fields of complex organic material settled from high altitude (*2*). A variety of cryovolcanic surface features can be attributed to Titan's recent internal activity (*3*). The isotopic signature of carbon and nitrogen and the presence of $^{40}Ar$ in Titan's atmosphere suggests relatively late formation of at least part of Titan's atmosphere by cryovolcanic degassing of the ice-rock interior (*4*, *5*).

Titan and most other satellites are in synchronous rotation and subject to tidal forces exerted by their primaries. The nonspherical part of their gravity fields is predominated by the spin and tidal contributions, which can be determined from the polar oblateness and equatorial ellipticity of the gravity field, respectively. Iess *et al*. determine both contributions by using different flyby geometries. Titan's ratio of the spin and tidal coefficients is close to 10:3, a value consistent with theoretical predictions for a synchronously rotating satellite in hydrostatic equilibrium. This allows Iess *et al*. to deduce Titan's axial moment of inertia with great precision, thereby providing a measure for the concentration of mass toward the center. Taken together with the satellite's known mean density, interior structure models can then be constructed. The new results imply that Titan's state of internal differentiation (see the figure) is an intermediate between partly differentiated (or separated) Callisto and Ganymede. The latter is strongly differentiated into an iron-rich liquid core, surrounded by a silicate rock mantle and overlain by a water ice/liquid shell and possesses a self-sustained, dipolar magnetic field (*6*).

The interpretation of Titan's gravitational field in terms of interior structure leads Iess *et al*. to conclude that either incomplete separation of the primordial mixture of ice and rock from which Titan formed or the presence of a substantially hydrated rock-rich central core could explain the inferred axial moment of inertia. Both interpretations differ from interior models considered previously (*5*, *7*–*9*), with strong implications for Titan's thermal history. The implication is that Titan's interior may have failed to get sufficiently hot for melting of a substantial portion of the primordial ice-rock mixture to occur and separation of ice from rock to proceed. There is notable discrepancy between the (hydrostatic) gravity field and the large-scale topography of Titan, as deduced from Cassini radar altimetry (*10*). Titan is slightly oblate, so that its poles have lower elevations than the equator, causing associated gravity anomalies which are attributed by Iess *et al*. to the presence of warm ice below. Thermodynamic models (*5*, *7*–*9*) suggest the present existence of a subsurface water ocean, sandwiched between a stagnant, floating ice shell and the dense interior, similar to those proposed for Ganymede and Callisto. Nonpolar solutes like ammonia and methanol and dilute salty impurities would lead to an appreciable melting point depression of the ice, resulting in even thicker and colder subsurface oceans. It is an open issue, however, how such oceans could have survived on cold and incompletely differentiated icy satellites such as Callisto and, as we now know, Titan.

The results obtained by Iess *et al*. facilitate comparison between Titan, Ganymede, and Callisto and indicate that satellites of similar size and composition in terms of their primor-

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