The Copper-Enzyme Family of Dopamine β-Monoxygenase and Peptidylglycine α-Hydroxylating Monooxygenase: Resolving the Chemical Pathway for Substrate Hydroxylation*

Published, JBC Papers in Press, November 21, 2005, DOI 10.1074/jbc.R500011200
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Dopamine-β-monoxygenase (DBM)2 and peptidylglycine α-hydroxylating monooxygenase (PHM) belong to a small class of copper proteins found exclusively in higher eukaryotes. These physiologically important enzymes catalyze the transformation of dopamine to norepinephrine (DBM) (Equation 1) and C-terminal glycine-extended peptides to their α-hydroxylated products (PHM) (Equation 2). Although their substrate specificities are grossly different, these enzymes greatly resemble each other in many other respects.

Both enzymes are localized in subcellular compartments: the chromaffin vesicles of the adrenal gland or synaptic vesicles of the sympathetic nervous system (DBM) (1) and the secretory vesicles of the pituitary gland (PHM) (2). Although DBM and PHM exist in soluble and membrane-bound forms within their vesicular compartments (3, 4), the majority of mechanistic studies have been completed with the more tractable soluble enzymes. The physiological roles played by soluble and membrane-bound enzymes may be different, but the chemical mechanisms are almost certain to be the same.

Sequence and Structural Features of DBM and PHM

The in vivo production of PHM is accompanied by the expression of a second activity that leads to the cleavage of the α-hydroxyepetide in Equation 2 to the biologically active, C-terminally carboxamidated peptide and glyoxylic acid (4). This second activity is found either in a separate enzyme (peptidylglycine α-amidating lyase) or within a single polypeptide chain that also contains DBM activity. Although DBM and PHM exist as soluble and membrane-bound forms within their vesicular compartments (3, 4), the majority of mechanistic studies have been completed with the more tractable soluble enzymes. The physiological roles played by soluble and membrane-bound enzymes may be different, but the chemical mechanisms are almost certain to be the same.

Solution Studies Indicate That DBM and PHM Are Mechanistically Interchangeable

A successful, high level expression system has never been established for DBM; therefore, detailed x-ray structural studies and site-specific isotope-labeling experiments have been applied to DBM and PHM, indicating an extraordinary conservation of properties.

Both enzymes have been shown to undergo a cyclical reduction of their copper sites by ascorbic acid, the physiologically relevant and preferred reductant (11, 12). The resulting Cu(I) sites are returned to the +2 valence state in the presence of substrate and dioxygen via a formal “ping-pong” mechanism in which reductant and substrates interact with different forms of enzyme that are separated by irreversible chemical processes. A ping-pong mechanism indicates that both electrons needed for the hydroxylation of substrate can be provided by the same enzyme. In support of such a mechanism, single or double freeze or acid quench studies of reduced DBM in the absence of exogenous reductant have demonstrated concomitant formation of Cu(II) and hydroxylated product in kinetically competent processes (11, 13). These properties require that the electron stored on CuM be capable of transferring to the CuH site at a rate compatible with catalytic turnover.

One of the major goals of investigation of DBM and PHM has been to isolate their chemical steps, such that detailed mechanistic information can be inferred regarding the nature of O2 and substrate activation. Use of deuterated substrates of comparable reactivity (dopamine and histidine with DBM and PHM, respectively) leads to relatively small kinetic isotope effects, indicating that substrate activation is only partially rate determining under steady state conditions. However, comparison of experimentally determined deuterium and tritium isotope effects has permitted calculation of the magnitude of deuterium isotope effects on the isolated C−H cleavage step (14–16). These “intrinsic” values for $k_o/k_d$ (Table 1) show remarkable agreement between DBM and PHM; despite their differing sequences and substrate specificities, both enzymes catalyze C−H abstraction reactions via identical activated complexes! These results reinforce the assumption, based on sequence comparisons, that DBM and PHM function via identical chemical mechanisms.

Also shown in Table 1 are the magnitudes of observed O−18 kinetic isotope effects using either H- or D-labeled substrates (14, 17). Although the measured values differ somewhat between DBM and PHM, consistent with somewhat different rate-limiting steps under steady state conditions, in both instances the magnitude of $k_{H-O}^d/k_{H-O}^a$ is seen to increase when substrate is deuterated. This highly significant observation demonstrates that the activation of both substrates and O2 must be fully connected by a reversible chemical process.

* This minireview will be reprinted in the 2006 Minireview Compendium, which will be available in January, 2007.

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2 The abbreviations used are: DBM, dopamine β-monoxygenase; PHM, peptidylglycine α-hydroxylating monooxygenase; DFPEA, N-acetyl, diido-Tyr-Gly, is shown to bind close to the CuM site, adjacent to Arg-240, implicating the CuM domain as the site of substrate hydroxylation. A recent x-ray structure of PHM with a slowly reacting substrate bound at the CuM site shows the presence of a bound O2 in close proximity to substrate as well (9). The structure of PHM reveals many striking and unexpected features. These include a lack of bridging ligands between the copper sites, consistent with earlier EPR studies that had failed to show any spin coupling between the paramagnetic copper centers (10). It is known that the two electrons consumed during substrate hydroxylation are stored in the CuM and CuH sites (11). Although it was conceivable that the metal centers could approach each other during the catalytic cycle, there is no structural evidence for a hinge region capable of facilitating such a movement. Although DBM and PHM belong to a multi-copper family of proteins, the copper sites appear to perform different functions, that of substrate hydroxylation (CuM) and electron storage/transfer (CuH). Perhaps the most startling feature to emerge from x-ray studies is the fully solvent-exposed nature of the copper sites, raising the questions of (i) how DBM and PHM carry out the regio- and stereospecific hydroxylations of Equations 1 and 2 and (ii) how they carry out controlled electron transfer from CuH to CuM through bulk solvent.

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**TABLE 1**

PHM and DβM proceed by identical transition states (Refs. 14, 15, and 17)

| Enzyme | Intrinsic $k_{\text{st}}/k_{\text{ct}}$ for C–H | Observed $k_{\text{st}}/k_{\text{ct}}$ for O–O |
|--------|--------------------------------|----------------------------------|
|        | $k_{\text{st}}/k_{\text{ct}}$ for C–H | $k_{\text{st}}/k_{\text{ct}}$ for O–O |
|        | 1° | 2° | 1° | 2° |
| DβM   | 10.9 | 1.19 | 1.0197 | 1.0256 |
| PHM   | 10.6 | 1.20 | 1.0167 | 1.0216 |

$^{a}$ Substrate for DβM is dopamine: $k_{\text{C-H}} = 1200$ s$^{-1}$; bond dissociation energy = 85 kcal/mol.

$^{b}$ Substrate for PHM is hippuric acid: $k_{\text{C-H}} = 810$ s$^{-1}$; bond dissociation energy = 87 kcal/mol.

The Challenge: How Can a Solvent-exposed Mononuclear Copper Center Perform Its Catalytic Function?

Hydrogen Transfer Is Non-classical—With the inescapable connection between C–H and O$_2$ activation in monooxygenase reactions, early studies focused on characterizing the properties of C–H activation. Structure-reactivity correlations on DβM, using a series of ring-substituted phenethylamines, indicated a slope of +1.5, i.e. a significantly faster rate with ring-donating substituents (15). This was originally interpreted in terms of an electron-deficient transition state resulting from hydrogen atom transfer between substrate and activated O$_2$. However, as elaborated below, the H-transfer reaction has been reformulated in the context of a modified Marcus model, whereby hydrogen transfer occurs quantum mechanically and trends in reactivity are expected to reflect the impact of reaction driving force on rate (cf. Ref. 18).

Four possible mechanisms for O$_2$ activation in DβM and PHM can be outlined for experimental testing. These range from the formation of a one-electron reduced intermediate (metal superoxo, Cu$_{\text{M}}$(II)(O$_2^-$)). Mechanism I, to two-electron reduced species (metal peroxo, Cu$_{\text{M}}$(II)(O$_2$)). Mechanism II, or metal hydroperoxo (Cu$_{\text{M}}$(II)(HO$_2$)). Mechanism III, and finally to a highly reduced Cu$_{\text{M}}$(II)(OH$_2$) formed via the reductive cleavage of Cu$_{\text{M}}$(II)(HO$_2$) by a conserved active site tyrosine. Mechanism IV.

One of the most puzzling observations on DβM came from measurements of O-18 discrimination in the uptake of O$_2$ using phenethylamine substrates. At the time of these measurements, it was generally assumed that a peroxo-type species, which had accepted one electron each from both Cu$_{\text{M}}$ and Cu$_{\text{H}}$, would be the functional oxidizing species (Mechanism II or III). With the expectation that there would be more bond cleavage at the O–O peroxo bond as the reaction became less favorable (later transition state), the O-18 isotope effect should have become larger with decreasing substrate reactivity. The experimental data indicated a decreasing magnitude for the O-18 kinetic isotope effect as the rate constant for C–H activation fell over 2.5 orders of magnitude (18). In an effort to reconcile the conflict between observation and prediction, a mechanism was put forth in which a copper hydroperoxo species reacted further with a conserved active site tyrosine near Cu$_{\text{M}}$ to yield the copper oxo species as the reactive intermediate (Mechanism IV). Although this mechanism was not easily tested with DβM, eventual preparation of the tyrosine mutant of PHM, Y318F, indicated no impact on $k_{\text{ct}}$ and, most significantly, less than a 4-fold reduction in the rate of C–H activation. This site-specific mutagenesis experiment effectively eliminated Mechanism IV from consideration (19).

The lack of agreement between experimental and predicted trends in O-18 isotope effects argued for a reassessment of underlying assumptions. Given the growing evidence for H-tunneling in enzyme systems, the most reasonable explanation seemed to lie with the presence of non-classical behavior in PHM and DβM; this would preclude a simple prediction of the "position of the H-transfer transition state" from trends in substrate reactivity. In fact, early evidence for "deviant behavior" in the DβM and PHM reactions had been evident from the comparison of the magnitudes of intrinsic primary and secondary hydrogen isotope effects (Table 1). Kinetic isotope effects that are formulated in the context of transition state theory are expected to yield similar conclusions about the structure of the transition state, using either intrinsic primary or secondary effects. Yet, the data in Table 1 imply a symmetrical transition state (from the 1° kinetic isotope effect) versus a late transition state (from the 2° kinetic isotope effect).

Definitive evidence for non-classical behavior in DβM and PHM became available from a detailed investigation of hydrogen isotope effects as a function of temperature. Recognizing that chemistry is unlikely to be rate-determining under the available experimental conditions, Francisco et al. (16) pursued methods for the very precise determination of primary deuterium and tritium isotope effects. From the measured isotope effects, the intrinsic primary hydrogen isotope effect on the isolated C–H cleavage step could be analyzed as a function of temperature, indicating little change across the experimental temperature range ($E_\text{a}(D) = E_\text{a}(H)$). By contrast, the semi-classical theory of isotope effects that incorporates the zero point vibrational energy of the reacting bond predicts that the isotope effect will depend on temperature and disappear in the high temperature regime. Numerous enzyme systems have now been documented to show a similar pattern of temperature-independent isotope effects, implicating a quantum mechanical transfer of hydrogen that is linked to the environment (20, 21).

Ruling Out the "Usual Suspects" for the Activated Oxygen Species—It is of considerable interest that the data for PHM are well fit using a modified Marcus-like model (16). In the course of modeling the temperature behavior of the isotope effects, it was necessary to "parse" the experimentally observed enthalpy of activation into steps that involve oxygen activation prior to hydrogen transfer. Given the requirement that these steps be reversibly connected, the most plausible hypothesis was that activated oxygen is formed in an uphill process followed by a second uphill, rate-limiting H-transfer.

The large driving force for formation of H$_2$O$_2$ from O$_2$, suggested that the Cu$_{\text{M}}$(II)–OOH (Mechanism III) may not be a good candidate for the activated O$_2$ species in DβM and PHM. As an experimental approach to test for the presence of Cu$_{\text{M}}$(II)–OOH, it was reasoned that formation of Cu$_{\text{M}}$(II)–OOH at the highly solvent-exposed active site of PHM/DβM would lead to some loss of H$_2$O$_2$ in the presence of substrates of greatly reduced intrinsic reactivity. This was initially examined with DβM, using substrates that vary by 2 $\times$ 10$^{-3}$-fold in reactivity. As summarized in Ref. 22, measurement of the ratio of O$_2$ consumption to product formation indicated tight coupling for all conditions. Similarly, using wild type PHM and a mutant at the Cu$_{\text{M}}$(II) site (H72A) that retains its ability to bind copper while reducing $k_{\text{cat}}$/K$_{\text{M}}$(O$_2$) almost 10$^3$-fold, no diminishment in the degree of coupling between O$_2$ and substrate consumption was observed. This behavior is in stark contrast to other enzymes known to form metal hydroperoxide intermediates, where uncoupling of O$_2$ reduction and substrate hydroxylation are common observations (cf. Refs. 24 and 25). In addition to eliminating a metal hydroperoxide from consideration, the available data also appear to rule out a mechanism in which O$_2$ was proposed to bind initially to Cu$_{\text{H}}$ to form a Cu$_{\text{M}}$(II)(O$_2$) intermediate, followed by dissociation of the superoxide anion and its migration across the solvent interface to bind and be further reduced at Cu$_{\text{M}}$(II). Analogue to a Cu$_{\text{M}}$(II)–OOH inter-

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mediate, some degree of uncoupling of \( O_2 \) uptake and substrate utilization would be expected for a mechanism of this type.

We now turn our attention to the Cu(II)-peroxy anion as an oxygenating species, Mechanism II, noting that, in the absence of a proton, the dianionic \( O_2^- \) may be expected to be tightly bound at CuMe. Although early kinetic studies on DJM had pointed toward a role for general acid catalysis in \( O_2 \) activation, the x-ray structure of PHM failed to reveal an active site functional group capable of such behavior. This led naturally to the proposal of a deprotonated copper hydroperoxide anion as the catalytic species (8). One key feature of CuMe(II)(\( O_2^- \)) is the prediction that both coppers will undergo oxidation in the course of \( O_2 \) reduction to CuMe(II)(\( O_2^- \)). Because the copper centers are expected to remain at a long distance (~10–11 Å) in the intermediate complex and, hence, uncoupled, it should be possible to observe the Cu(II) EPR signals in a suitably designed experiment. To this end, a non-reactive analog of phenethylamine, \( \beta,\beta \)-difluorophenethylamine (DFPEA), was synthesized and reacted with DJM in a rapid mixing device (22). Unlike a phenethylamine substrate that leads to a time-dependent formation of Cu(II) that correlates with product formation, little oxidation of the copper centers was observed with the difluoro analog on the time scale of the experiment (22). Although it is impossible to rule out non-productive binding of DFPEA as well as the formation of such a low level of CuMe(II)(\( O_2^- \)) that it is EPR-undetectable, neither of these explanations is very satisfactory. A third possibility, the formation of an EPR silent oxygen-activated species in the presence of DFPEA, begins to look very attractive.

**Proposal of Copper-Superoxo as the Active Oxygen Species in DJM and PHM**

Mechanism I, the formation of a copper-superoxo intermediate, appears to provide a working mechanism that is capable of rationalizing the voluminous amount of data available for DJM and PHM. The expanded mechanism of Fig. 2 also allows an answer to the long standing question of how these enzymes catalyze electron transfer across bulk water at a rate that is compatible with catalytic turnover.

Starting with the fully reduced enzyme on the upper left-hand side of Fig. 2, substrate and \( O_2 \) bind to produce the ternary complex. This is the trigger for initial \( O_2 \) activation involving electron transfer from CuMe(I) to \( O_2 \) to form the EPR silent copper-superoxo intermediate. The latter is believed to be generated in an energetically uphill process, consistent with the impact of substrate deuteration on the \( O_18 \) isotope effects and the fact that oxygen uptake and product formation are so tightly coupled. Recent density functional theory calculations support such an energetic view of CuMe(II)(\( O_2^- \)) in DJM/PHM (27). Within the protein active sites, the reactivity of the Cu(II)-superoxo species is expected to be tightly linked to the degree of charge transfer from metal to \( O_2 \) together with the tightness of binding of the resultant superoxide anion to Cu(II). Subsequent transfer of a hydrogen atom from substrate via tunneling will also be linked to the ability of the protein to sample many different conformational substates; only a subset of these configurations is expected to possess the requisite energetic and internuclear distance requirements that can give rise to efficient wave function overlap from the hydrogen in the donor substrate to acceptor oxygen (cf. Ref. 21). These properties suggest that a great deal of “subtle tuning of reactivity” is at work within the active sites of DJM and PHM.

One of the more gratifying aspects of the mechanism in Fig. 2 concerns the stage at which the second electron from CuMe(I) enters into the reaction mechanism. The rate constant for the C–H bond cleavage step with the natural substrate dopamine in DJM is almost \( 10^3 \) s\(^{-1} \) (16). If electron transfer from CuMe to CuMe preceded the substrate activation step, it would have to occur significantly faster than \( 10^3 \) s\(^{-1} \). Many authors have debated how the electron could move this quickly through bulk water, proposing pathways that involve portions of the protein and/or the substrate (e.g. Refs. 28 and 29). Experimental testing of these proposals has, thus far, failed to provide support for their existence. In the context of Fig. 2, the second electron does not transfer to the CuMe site until after an irreversible hydrogen atom transfer, placing the long range electron transfer into \( k_{ee} \) which is a much slower process, ~40 s\(^{-1} \) (28). This not only relaxes the kinetic constraints for the electron transfer step but alters its thermodynamic driving force. In the original mechanism for DJM proposed by Evans et al. (22), the intermediate CuMe(III)–\( O_2H^- \) is proposed to undergo reductive cleavage by CuMe via \( k_{cr} \) to produce water and a CuMe(II)-oxo
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radical, which then rapidly recombines with the substrate-derived radical to give an inner sphere alcohol product. The computations of Solomon and coworkers (27) suggest an alternative in which the substrate-derived radical abstracts hydroxyl radical directly from the CuM(II)–OOH to form unliganded product; the resulting CuM(II)-oxo species then undergoes reduction by CuH to CuM(II)–OH in a thermodynamic favorable process. One feature of the DJBM mechanism that may favor the inner sphere product complex shown in Fig. 2 is the observation that \( k_{cat} \) is faster with phenethylamine substrates containing electron-withdrawing substituents. This has been used to argue for the intermediacy of an inner sphere alcohol product complex that undergoes (partially) rate-limiting dissociation to free product as part of the \( k_{cat} \) process (15).

Concluding Comments

The extensive investigations of the DJBM/PHM systems represent a “historical saga,” in which the design and interpretation of early studies were dominated by existing paradigms such as the reactivity of binuclear metal complexes (30) or heme-iron chemistry (31). We have reached a new stage where these bizarre and beautiful enzymes have begun to reveal their unique chemistry. Many experimental challenges remain, which include the precise tuning of the active site for hydrogen transfer and the possible participation of regions of the protein distal from the active site in this process. The vexing question of the exact mechanism of long range electron transfer between the CuH and CuM sites also awaits elaboration.

Acknowledgments—I thank Dr. Corinna Hess for insightful reading and comments, and the numerous graduate students and postdoctoral fellows who have contributed their energy and creativity to these studies.

REFERENCES

1. Stewart, L. C., and Klinman, J. P. (1988) Annu. Rev. Biochem. 57, 551–591.
2. Eipper, B. A., Stoffers, P. A., and Mains, R. E. (1992) Annu. Rev. Neurosci. 15, 57–85.
3. Winkler, H., and Carmichael, S. W. (1982) in The Secretory Granule (Poinsner, A. M., and Trifaro, J. M., eds) Elsevier Biomedical Press, Amsterdam.

4. Prigge, S. T., Mains, R. E., Eipper, B. A., and Amzel, L. M. (2000) Cell. Mol. Life Sci. 57, 1236–1259.
5. Kulathila, R., Merkler, K. A., and Merkler, D. J. (1999) Nat. Prod. Rep. 16, 145–154.
6. Lamoroux, A., Vigny, A., Faucon Biguet, V., Darmon, M. C., Frank, R., Henry, J. P., and Mallet, J. (1987) EMBO J. 6, 3931–3937.
7. Southan, C., and Kruse, L. I. (1989) FEBS Lett. 255, 116–120.
8. Prigge, S. T., Kolhekar, A. S., Eipper, B. A., Mains, R. E., and Amzel, L. M. (1999) Nat. Struct. Biol. 6, 976–983.
9. Prigge, S. T., Eipper, B. A., Mains, R. E., and Amzel, L. M. (2004) Science 304, 864 – 867.
10. Klinman, J. P. (1996) Chem. Rev. 96, 2541–2561.
11. Brenner, M., and Klinman, J. P. (1989) Biochemistry 28, 4664–4670.
12. Freeman, J. C., Villafranca, J. J., and Merkler, D. J. (1993) J. Am. Chem. Soc. 115, 4923–4924.
13. Brenner, M., Murray, C. J., and Klinman, J. P. (1989) Biochemistry 28, 4656–4664.
14. Francisco, W. A., Merkler, D., Blackburn, N., and Klinman, J. P. (1998) Biochemistry 37, 8244–8252.
15. Miller, S. M., and Klinman, J. P. (1982) Biochemistry 21, 3091–3096.
16. Francisco, W. A., Knapp, M. J., Blackburn, N. J., and Klinman, J. P. (2002) J. Am. Chem. Soc. 124, 8194–8195.
17. Tian, G., Berry, J. A., and Klinman, J. P. (1994) Biochemistry 33, 226–234.
18. Knapp, M. J., Rickert, K., and Klinman, J. P. (2002) J. Am. Chem. Soc. 124, 3865–3874.
19. Francisco, W. A., Blackburn, N. J., and Klinman, J. P. (2003) Biochemistry 42, 1813–1819.
20. Klinman, J. P. (2006) Biochim. Biophys. Acta, in press.
21. Knapp, M. J., and Klinman, J. P. (2002) Eur. J. Biochem. 269, 3113–3121.
22. Evans, J. P., Ahn, K., and Klinman, J. P. (2003) J. Biol. Chem. 278, 49691–49698.
23. Eipper, B. A., Quon, A. S. W., Mains, R. E., Boswell, J. S., and Blackburn, N. J. (1995) Biochemistry 34, 2857–2865.
24. Solomon, E. I., Brunold, T. C., Davis, M. I., Kemsley, J. N., Lee, S.-K., Lehnert, N., Neese, R., Skulan, A. J., Yang, Y.-S., and Zhou, J. (2000) Chem. Rev. 100, 235–349.
25. Gorsky, L. D., Koop, D. R., and Coon, M. J. (1984) J. Am. Chem. Soc. 106, 2541–2561.
26. Solomon, E. I., and Coon, M. J. (1984) J. Am. Chem. Soc. 106, 4991–5000.
27. Bell, J., El Meslini, R., D’Amato, D., Mains, R. E., and Eipper, B. A. (2003) Biochemistry 42, 7133–7142.
28. Chen, P., and Solomon, E. I. (2004) J. Am. Chem. Soc. 126, 13168–13169.
29. Francisco, W. A., Wille, G., Smith, A. J., Merkler, D. J., and Klinman, J. P. (2004) J. Am. Chem. Soc. 126, 8241–8287.