

# The Electronic Nature of the Peptidylglycine $\alpha$ -Amidating Monooxygenase Transition State: Evidence for a Radical Mechanism

*Edward W. Lowe Jr. & David J. Merkler*

*Department of Chemistry, University of South Florida, Tampa, Florida, United States*

Peptidylglycine  $\alpha$ -amidating monooxygenase (PAM) is a copper and zinc-dependent, bifunctional enzyme that catalyzes the cleavage of glycine-extended peptides to the corresponding amides and glyoxylate. The sequential action of hydroxylating the glycyl  $\alpha$ -carbon and then cleaving the carbonamide bond are dependent upon the peptidylglycine  $\alpha$ -hydroxylating monooxygenase (PHM) and peptidylglycine  $\alpha$ -amidating lyase (PAL) domains, respectively. PAM is responsible for activating peptide prohormones in vivo. This study attempts to demonstrate radical formation during catalysis by examining a series of ring-substituted 4-phenyl-3-butenoates (PBA) as mechanism-based inhibitors for PAM. All the substituted PBAs examined inactivate the enzyme under turnover conditions requiring O<sub>2</sub>, copper, and ascorbate. The determination of the partition ratios and inactivation kinetics of these ring-substituted PBAs has allowed for the determination of  $k_{inact}/K_{O_2}$ , the log of which has been compared against  $\sigma^+$  yielding a negative slope ( $\rho$  value). These findings provide evidence for a radical mechanism and are consistent with a similar analysis of the inactivation of D $\beta$ M by ring-substituted 3-phenylpropenes.