Does the strong base in the vitamin K carboxylation of Glu residues actually exist? A free radical mechanism for γ-carboxylation

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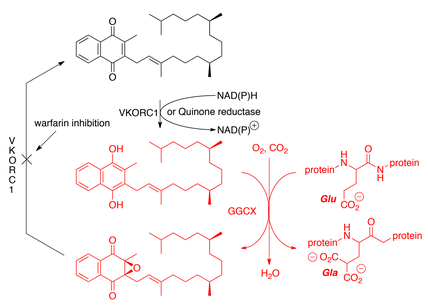
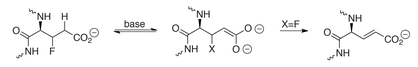
Background

The vitamin K cycle

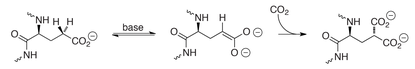


What is known about this reaction?

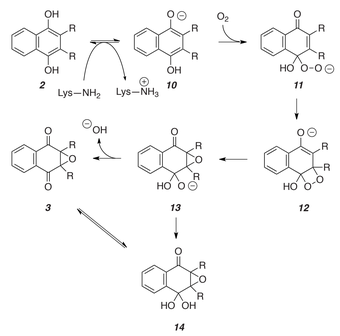
The carboxylation of a substrate analog containing a 3-fluoroglutamate residue leads to loss of hydrogen fluoride. The treatment of a similar substrate analog (without the fluorine) in tritiated water under conditions of CO_2 starvation leads to stereospecific incorporation of tritium into the Glu side chain.



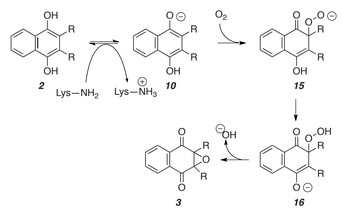
Conclusion: there is a very strong base generated in the reaction, and this base deprotonates the Glu side chain to generate a carboxylate dianion that reacts with CO_2 to give the carboxylated (Gla) residue.



The Dowd base strength amplification mechanism



The Zheng and Bruice mechanism

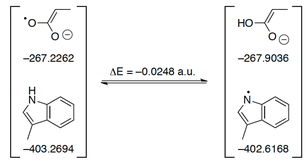


The primary difference between this mechanism and the Dowd base strength amplification mechanism is in the product of the oxygenation of the naphthyloxide anion. In the Zheng and Bruice mechanism, the attack of the anion on oxygen is at the 2-position; in the Dowd base strength amplification mechanism, it is at the 4-position. Both generate hydroxide anion in a highly hydrophobic environment.

The importance of Trp 157

The mutation W157R reduces the rate of carboxylation of the natural substrate, and model substrates by a factor of approximately 12.5, which indicates that this amino acid residue plays an important part in the mechanism of the reaction.

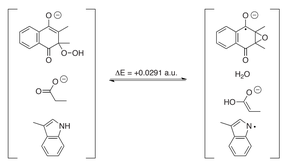
Hydrogen atom transfer to tryptophan



The reaction between the propionate radical anion and indole to give the hydroxyenolate anion derived from propionic acid and the indolyl radical is exothermic by just 0.0248 a.u., or 15.6 kcal mol^{-1}.

Does this also work from the Zheng and Bruice hydroperoxide?

Tryptophan-assisted hydrogen atom transfer can occur during the homolysis of the Zheng and Bruice hydroperoxide to give the same hydroxyenolate and water.

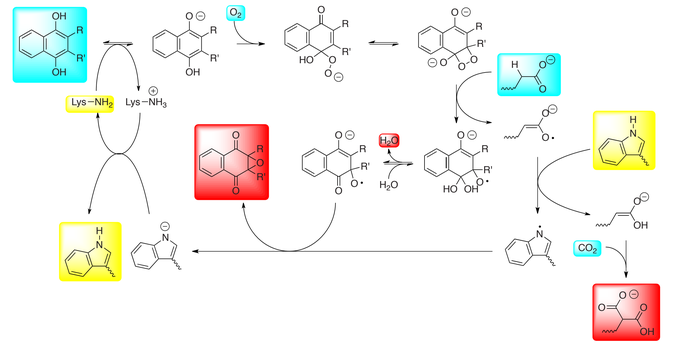


This reaction is endothermic by +0.0291 a.u., or 18.3 kcal mol^{-1}.

Conclusions about the mechanism

Two viable radical mechanisms

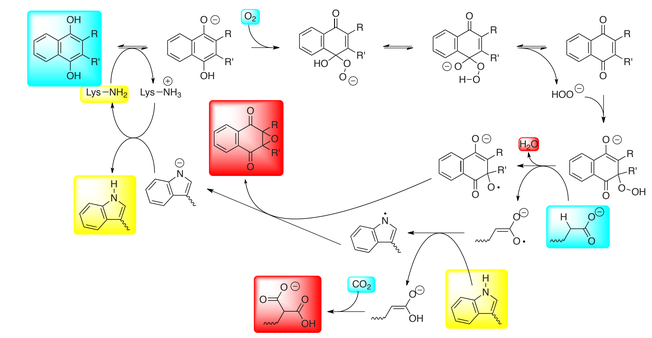
via the Dowd dioxetane



This free radical alternative addresses several unresolved problems in the Dowd base strength amplification mechanism:

- It removes the necessity for a strong base in the carboxylation reaction.
- It provides a mechanism for the involvement of free radicals in the mechanism that does not require the contra-thermodynamic carboxylation of an alkyl radical.
- It generates a hydroxyenolate anion by hydrogen atom transfer from the indole ring of a tryptophan side chain, thus:
  - providing a mechanism for Trp 157 to be an important part of the active site of the enzyme
  - describing a reaction pathway that is essentially thermoneutral or exothermic after the highly endothermic first step.

via the Zheng and Bruice hydroperoxide



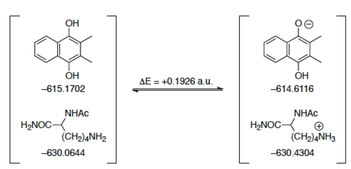
This free radical alternative to the Zheng and Bruice mechanism mechanism also addresses the same unresolved problems of the existing mechanism.

Computational results

Computations were carried out at the B3LYP/6-311++G(2df,2p) level. Vitamin K and its derivatives were modeled using 2,3-dimethyl-1,4-naphthalenediol as the base structure. The protein side chains were modeled as follows:

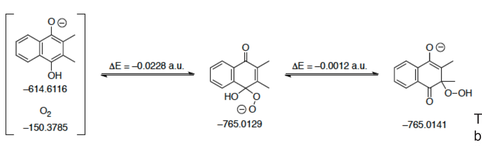
glutamate side chain was modeled using propanoate anion;
tryptophan was modeled using 3-methylindole;
lysine was modeled using *N*-acetyllysinamide.

Step 1. Deprotonation of the naphthalenediol



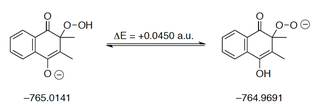
The gas-phase deprotonation is very endothermic. The hydrogen-bonded pair is the computational minimum.

Step 2. Reaction of the anion with molecular dioxygen



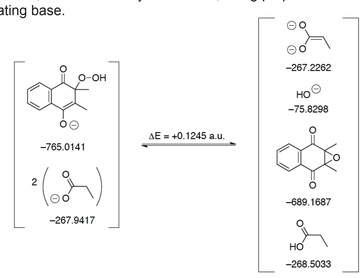
The reaction with oxygen at the 4-position is favorable by 0.0220 a.u. (13 kcal mol^{-1}). This initial hydroperoxide anion undergoes a proton transfer to give the 4-alkoxide, which is computationally unstable with respect to disproportionation to the complex of HO_2^- and the quinone. The addition of the hydroperoxide anion to the quinone is almost thermoneutral (exothermic by 0.0012 a.u., or 0.75 kcal mol^{-1}).

Step 3. Isomerization of the oxyanion



The isomerization of the enolate to the enol is endothermic by 0.0450 a.u., or 28.2 kcal mol^{-1}.

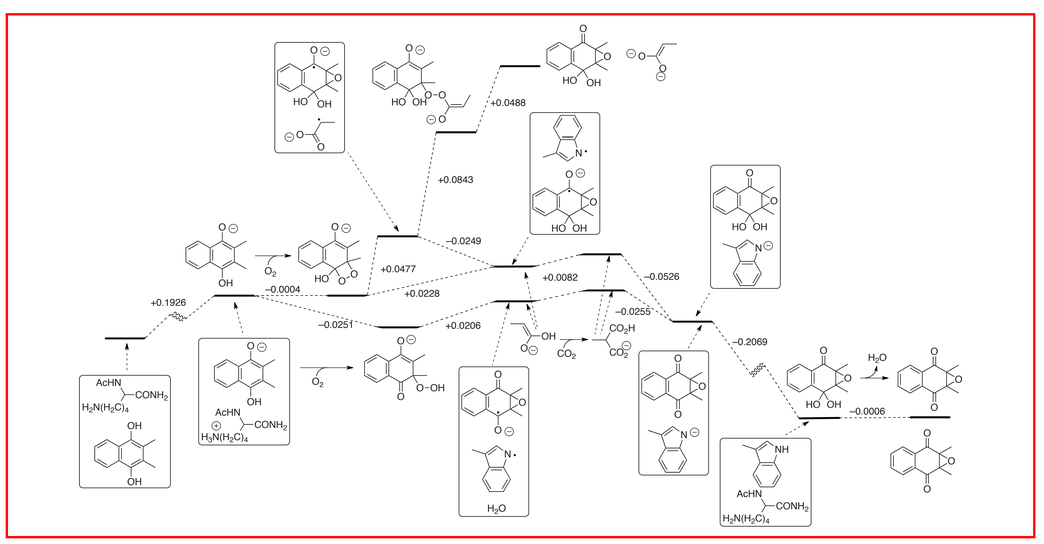
Step 4. Alternative, concerted generation of the quinone epoxide, hydroxide anion, and the carboxylate dianion, using propionate anion as the initiating base.



The concerted formation of hydroxide anion, however, is endothermic by 0.1245 a.u., or 78.1 kcal mol^{-1}. This is prohibitive, and suggests that this pathway is not truly viable.

Involving a Trp side chain has a dramatic effect on the overall course of the reaction. The products are the same, except that the propionate dianion becomes the hydroxyenolate anion, and the Trp is converted to its conjugate base. This reaction is still endothermic. but now by only 0.0200 a.u., or 12.6 kcal mol^{-1}.

Acknowledgments

We are pleased to acknowledge the financial support of this project by WiSys Technology Foundation, and the University of Wisconsin-Eau Claire Office of Research and Sponsored Programs.

Putting it all together: a combined energy profile for the radical mechanisms