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

The Dowd base strength amplification mechanism

The Zheng and Bruice mechanism

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 CO₂ elevation 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 anion that reacts with CO₂ to give the carboxylated (Gla) residue.

The importance of Trp 157

The mutation W157F 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.

Conclusions about the mechanism

Two viable radical mechanisms via the Dowd dioxetane

Does this also work from the Zheng and Bruice hydroperoxide?

Tryptophan-activated hydrogen atom transfer can occur during the homolytic cleavage of the Dowd and Bruice hydroperoxide to give the same hydroxyl- and water.

This reaction is endothermic by +0.0286 a.u., or 15.6 kcal mol⁻¹.

Computational results

Calculations were carried out at the B3LYP/3-311+G(2d,p) level. Vitamin K and its derivatives were modeled using 2,3-dimethyl-1,4-naphthoquinone as the base structure. The protein side chains were modeled as follows: glutamate side chain was modeled using propionate anion; tryptophan was modeled using 3-methylindole; tyrosine was modeled using N-acetyltyrosine.

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 oxygen

The reaction with oxygen at the 4-position is favorable by 0.0220 a.u. (13 kcal mol⁻¹). The initial hydroperoxide anion undergoes a proton transfer to give the 4-oxide, which is computationally unstable with respect to disproportionation to the complex of HO₂⁺ and the quinone. The addition of the hydroperoxide anion to the quinone is almost thermoneutral (endothermic by 0.0012 a.u., or 0.75 kcal mol⁻¹).

Step 3. Isomerization of the oxygen.

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 anion, using propionate anion as the initiating base.

The concerted formation of hydroxide anion, however, is exothermic 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 anion becomes the hydroxylate anion, and the Trp is converted to its conjugate base. This reaction is adiabatic, but now by only 0.0200 a.u., or 12.8 kcal mol⁻¹.

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

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