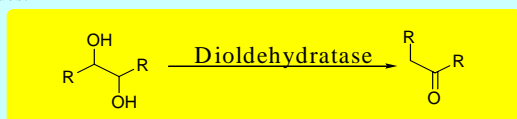


Design and Synthesis of Substrates to Probe Radical and Radical Ionic Intermediates in Coenzyme B12 Dependent Enzymes Such as Diol and Glycerol Dehydratase.

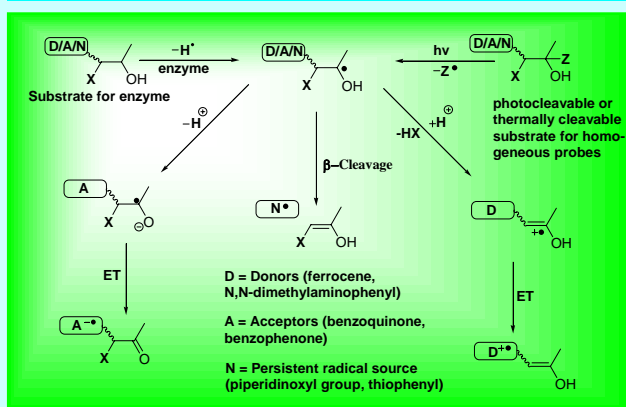
Introduction:

Since the isolation of Vitamin B₁₂ by Folkers¹, it has been found to serve as a cofactor in many enzymatic reactions that include carbon skeleton rearrangements, heteroatom eliminations and intramolecular amino group migrations.² Diol dehydratase is one such enzyme converting vicinal diols into aldehydes.^{3,4}



Our Approach:

- Translating short-lived radical and radical ionic intermediates into relatively long-lived ones either via fast β -cleavage or electron transfer process⁵ (ET process) directly at the active site of the enzyme.

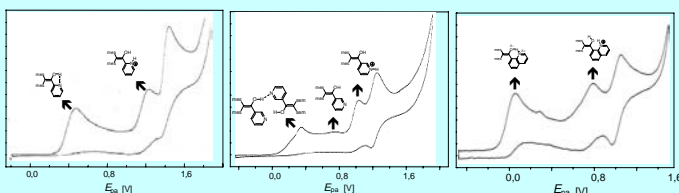


Advantages: Easy, efficient and rapid detection of radical and radical ionic intermediates

What factors can potentially influence the ET process at the active site of the enzyme?

- Hydrogen bonding at the active site of enzyme is commonly observed and utilised by the enzymes to anchor and preorient substrates, transition states and products during enzymatic action.

- Using cyclic voltammetry we have been able to demonstrate that hydrogen bonding can indeed modulate reduction or oxidation potentials of precursors of ketyl radical anion^{6,7,8} (~300 mV) and enol radical cations^{9,10} respectively (~500 mV).

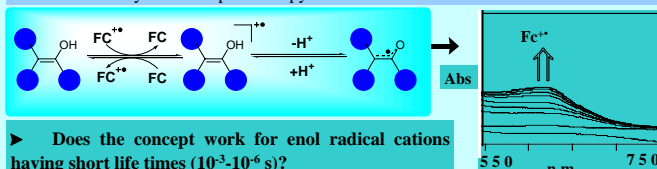


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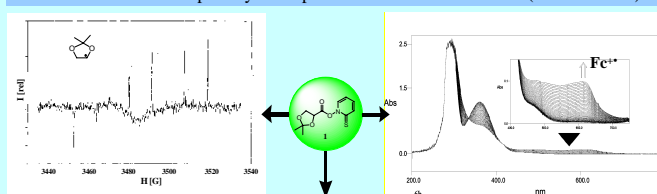
Does the ET concept work? How can we test it in solution?¹¹

- The concept was tested by intermolecular trapping of enol radical cations with a life time in the millisecond regime¹² using ferrocene as the donor. The whole process was monitored by UV-Vis spectroscopy.

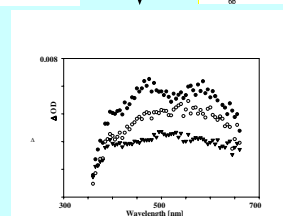


Does the concept work for enol radical cations having short life times (10⁻³-10⁻⁶ s)?

- In order to generate enol radical cations having life times ranging between 10⁻³-10⁻⁶ s Barton ester **1** was photolysed in presence of trifluoroacetic acid (and ferrocene).



- Radical formed after decarboxylation of Barton ester **1** during photolysis as observed by time resolved EPR

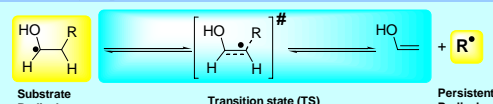


- Easy monitoring of trapping process using UV-Vis spectroscopy by photolysis of Barton ester **1** in presence of ferrocene and trifluoroacetic acid.

- LFP experiments performed on Barton ester **1** confirmed the formation of enol/enol ether radical cation¹³ with λ_{max} around 580 nm, which was quenched by addition of methanol. Thus even short-lived enol/enol ether radical cations can be trapped by ET process.

How fast are the β -cleavage processes?

- Theoretical calculations were performed on model compounds to get an overview of β -cleaving ability of various groups.



R group	Activation Energy [Kcal/mol]	Exo/Endothermicity of reaction [Kcal/mol]	Vibrational frequency of TS (cm ⁻¹)
-ONMe ₂	11.0	-6.8	505
-NO	1.2	-20.9	624
-SPh	0.5	-1.5	191
-SePh	2.8	-0.2	261
-SO ₂ Me	4.3	-4.1	283
-SO ₂ Ph	3.4	-5.0	275

Gaussian 98, UB3LYP/6-31G(d, p)

Acknowledgments:

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