Transition state stabilization in enzyme catalysis: insights into mechanism from QM/MM modelling

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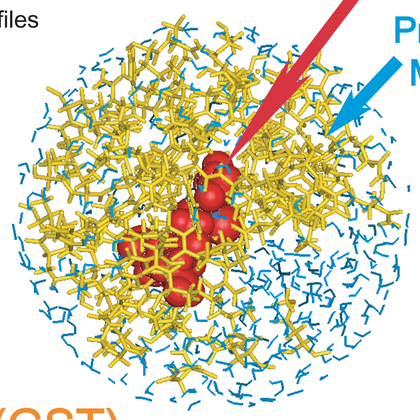
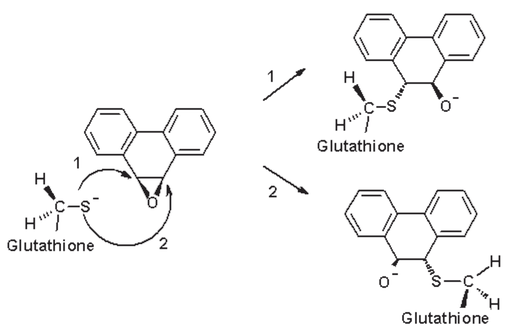
Introduction

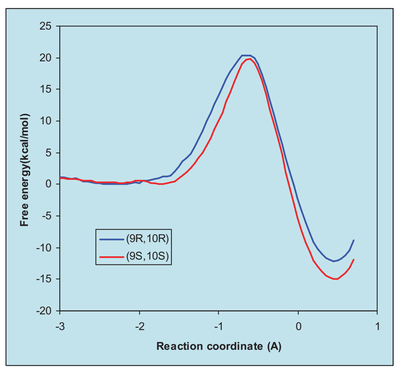
- Combined quantum mechanics/molecular mechanics (QM/MM) methods allow modelling of reactions within enzymes
- Model transition states and intermediates; identify and analyse key interactions
- Calculations with ab initio, semiempirical or density functional QM
- Molecular dynamics simulations for free energy profiles
- Small active site region treated QM (e.g 50 atoms)
- Surrounding protein and solvent (e.g. 25A radius) treated by MM (e.g. CHARMM)
- Test mechanistic proposals
- Test and predict effects of mutations
- Recent applications demonstrate the useful insight QM/MM modelling can provide: glutathione transferase (GST), chorismate mutase, and para-hydroxybenzoate hydroxylase (PHBH)



Glutathione S-Transferase (GST)

- Catalyses the addition of the tripeptide glutathione to endogenous and xenobiotic substrates
- Plays an important role in the detoxification and metabolism of xenobiotics (e.g. drugs)
- Exist as five classes (α, μ, π, κ, and θ) within which various isoenzymes are present
- Epoxide ring-opening of phenanthrene 9,10-oxide in M1-1 isoenzyme
- Two possible alternative diastereomeric producs can be formed (R,R) and (S,S)
- An important model reaction for understanding determinants of stereospecificity in GSTs

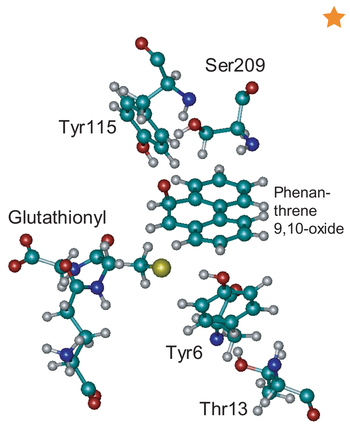




Calculated GST free energy profiles

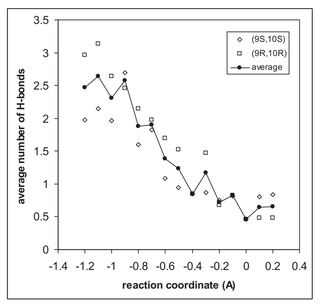
GST: QM/MM Dynamics Simulations

- Substrate and glutathione modelled QM with reaction-specific AM1 parameters
- Umbrella sampling MD along approximate reaction coordinate (r(C-O)-r(S-C))
- Successive simulations every 0.1A along reaction coordinate (e.g. 540ps total)
- WHAM (Weighted Histogram Analysis Method) used to give Free Energy Profile
- Free energy barrier is ~20 kcal/mol, compares well with experimental activation energy of ~18 Kcal/mol



GST active site (TS complex)

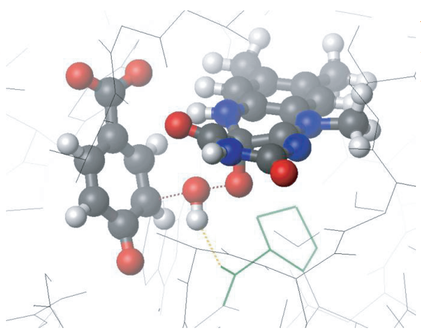
GST: solvation of thiolate sulfur along reaction path



GST: Mutations and stereospecificity

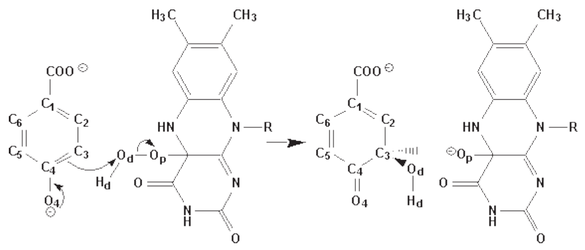
- M1-1 isoenzyme is not stereospecific: produces 50:50 mixture of (R,R) and (S,S) products (experiment again agrees with simulation findings)
- M2-2 isoenzyme produces only (S,S) product - why?
- Model effects of mutations (e.g. Ser209Ala, Asn8Asp and Thr13Ala represent differences between M1-1 and M2-2 GST isoenzymes)
- Calculate average changes in barrier and reaction energy through dynamics
- Asn8Asp mutation identified as determinant of stereospecificity (increases tbarrier for forming the (9R,10R) product more than barrier for (9S,10S)
- Agrees with the fact that the M2-2 isoenzyme preferentially forms (9S,10S)
- Also identified role for Tyr115 in TS stabilization: important for catalysis
- Simulations can help understand and predict effects of polymorphism on GST specificity and stereospecificity

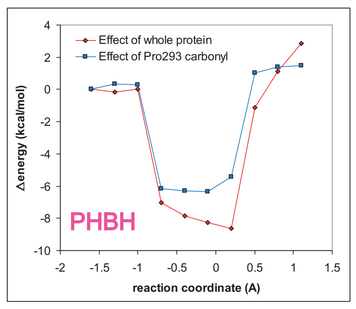
Para-hydroxybenzoate hydroxylase (PHBH)



PHBH active site showing transition state interaction with Pro293

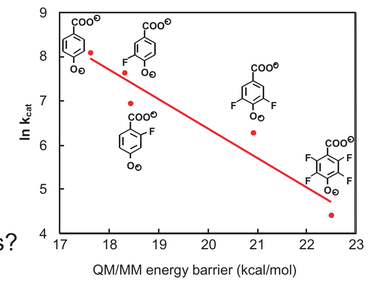
- Biodegradation of aromatic compounds (pollutants etc)
- Flavoprotein monoxygenase; hydroperoxyflavin intermediate
- Ab initio and semiempirical QM/MM
- Electrophilic aromatic substitution mechanism

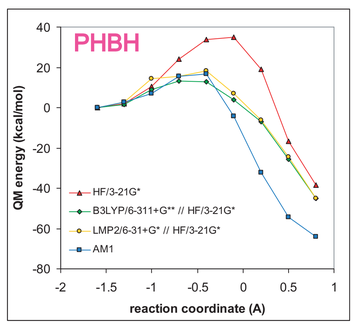




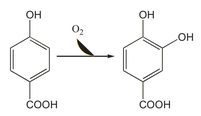
PHBH

- Calculations identified novel catalytic interaction
- Hydrogen bond with Pro293 carbonyl
- Specifically stabilizes transition state (only)
- Supported by subsequent experiments
- QM/MM tested by higher level calculations
- Now also found in phenol hydroxylase (PH)
- General feature of flavoprotein oxygenase catalysis?

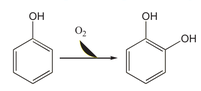


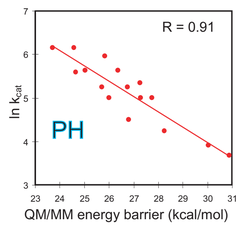


PHBH:



Phenol hydroxylase (PH):



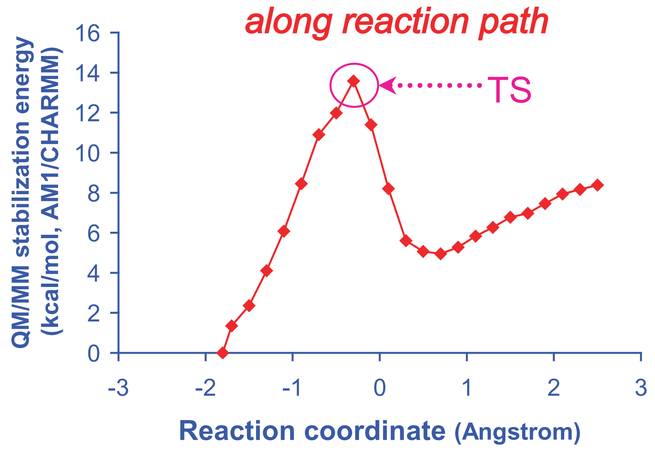


- Wide range of halogenated substrates
- Correlation between experimental and calculated barriers
- Validates QM/MM model
- Identification of mechanism
- Rate-limiting step
- Predict rates for alternative substrates
- Correlation with experimental rates for phenol hydroxylase also

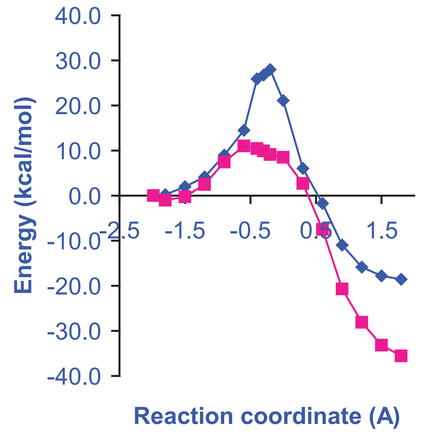
Chorismate mutase

- Claisen rearrangement of chorismate to prephenate
- No covalent catalysis by the enzyme
- Substrate strain/ the 'NAC' effect in catalysis?
- Ab initio and semiempirical QM/MM modelling
- Comparison with reaction in solution

Chorismate mutase: stabilization along reaction path

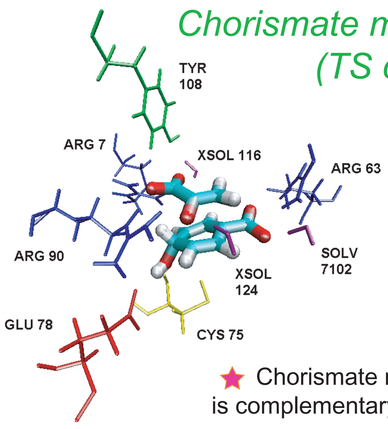


- Stabilization by the enzyme is greatest at the transition state
- Enzyme binds TS more strongly than substrate
- TS stabilization central to catalysis
- Substrate is distorted: strain/compression may also assist catalysis



- TS is stabilized (relative to substrate) more by the enzyme than it is in solution
- Calculated rate acceleration consistent with experiment

Chorismate mutase active site (TS complex)



- Chorismate mutase active site is complementary to the transition state
- Major contributions to TS stabilization from Arg90, Glu78, Arg7, and some bound water molecules

Conclusions

- QM/MM modelling can provide detailed insight into enzyme mechanisms
- Can compare mechanisms, model transition states
- Identify and analyse catalytic interactions
- Results demonstrate the importance of TS stabilization
- Novel TS stabilizing interaction identified in PHBH and PH
- Relative stabilities of TSs determine stereospecificity in GST
- Chorismate mutase active site is exquisitely well-organized to stabilize TS
- Modelling helps relate enzyme structure to activity

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