Chem 452 - Lecture 5 Catalytic Strategies 111028

Enzymes have evolved an array of different strategies or enhancing the power and specificity of the reactions they catalyze. For numerous enzymes the details have been worked out at the atomic level. In this lecture we will focus on four examples: chymotrypsin, carbonic anhydrase, the EcoRV restriction endonuclease, and myosin II ATPases.

Introduction

- Enzymes exhibit both catalytic power and specificity
- We will consider closely, four examples.

Introduction

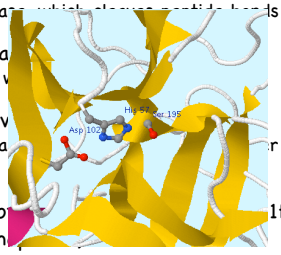
- Some Basic Catalytic Principles
  - Covalent Catalysis
  - General Acid/Base Catalysis
  - Catalysis by Approximation
  - Metal Ion Catalysis
  - Transition State Stabilization

Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO_2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic a
  - A Lyase, w
- EcoRV (1rv
  - A Hydrola r bonds in DNA
- Myosin mo 1fmw) 3.6.4.1 (Ch
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.



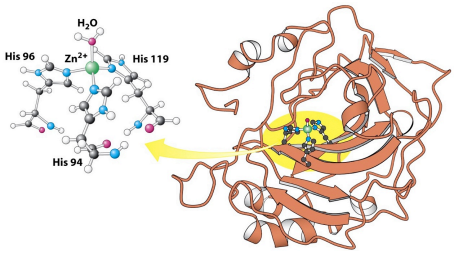
Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO_2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

Chem 452, Lecture 5 - Catalytic Strategies 4

Introduction

- Chymot
  - A Hydr
- Carboni
  - A Lyas
- EcoRV (
  - A Hydr DNA
- Myosin 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.



Chem 452, Lecture 5 - Catalytic Strategies 4

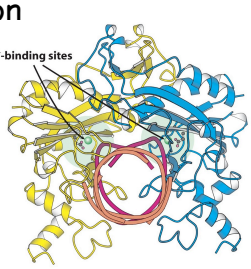
Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO_2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

Chem 452, Lecture 5 - Catalytic Strategies 4

Introduction

- Chymotrypsi
  - A Hydrolase proteins
- Carbonic anh
  - A Lyase, wh
- EcoRV (1rvb)
  - A Hydrolase nds in DNA



- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

Introduction

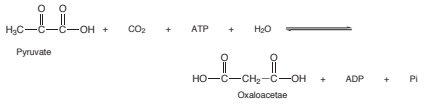
- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO_2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

Introduction

- Chymotrypsin (1gct) 3.4.21.1
  - A Hydrolase, which cleaves peptide bonds in proteins
- Carbonic anhydrase (1ca2) 4.2.1.1
  - A Lyase, which adds water to CO2.
- EcoRV (1rvb) 3.1.21.4
  - A Hydrolase, which cleave phosphodiester bonds in DNA
- Myosin motor domain ATPase (1fmv & 1fmw) 3.6.4.1 (Chapter 9)
  - An enzyme that couples the hydrolysis of ATP to the mechanical motion.

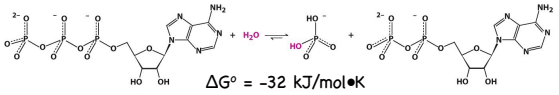
Myosin ATPase

- ATP is considered the energy currency of the cell.
  - The hydrolysis of ATP is used to drive many unfavorable processes
    - Reactions (ligases)
    - Transport across membranes
    - Mechanical motion
- For example, pyruvate carboxylase
  - Couples the hydrolysis of ATP to the formation of a carbon-carbon bond.



Myosin ATPase

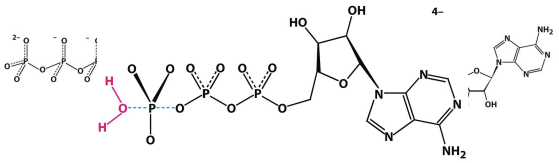
- Myosin ATPase couples the hydrolysis of ATP to mechanical motion.
  - The reaction is expected to have a pentavalent transition state intermediate



$\Delta G^{\circ} = -32$ kJ/mol•K

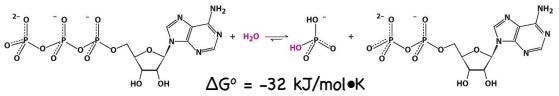
Myosin ATPase

- Myosin ATPase couples the hydrolysis of ATP to mechanical motion.
  - The reaction is expected to have a pentavalent transition state intermediate



Myosin ATPase

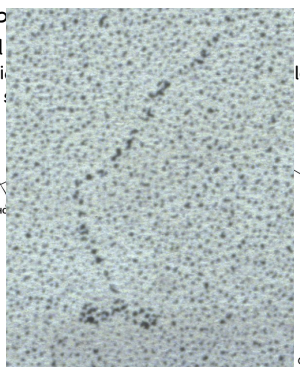
- Myosin ATPase couples the hydrolysis of ATP to mechanical motion.
  - The reaction is expected to have a pentavalent transition state intermediate



$\Delta G^{\circ} = -32$ kJ/mol•K

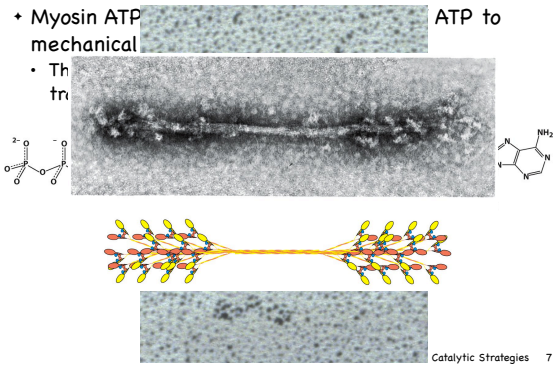
Myosin ATPase

- Myosin ATP ATP to mechanical
  - The reacti lent transition



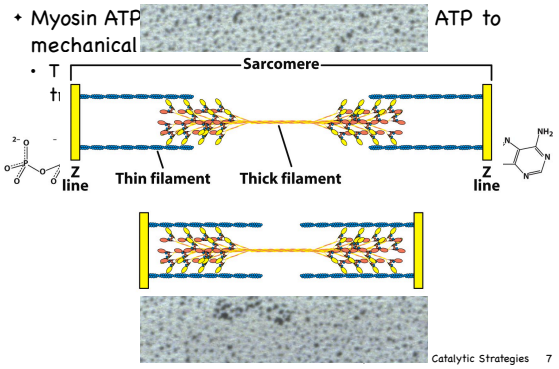
Myosin ATPase

- Myosin ATP ATP to mechanical
  - Th tr



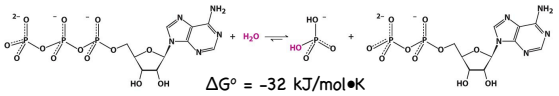
Myosin ATPase

- Myosin ATP ATP to mechanical
  - T th



Myosin ATPase

- Myosin ATPase couples the hydrolysis of ATP to mechanical motion.
  - The reaction is expected to have a pentavalent transition state intermediate

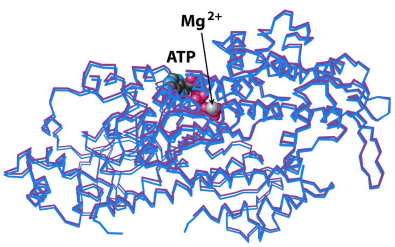


ΔG° = -32 kJ/mol•K

Myosin ATPase

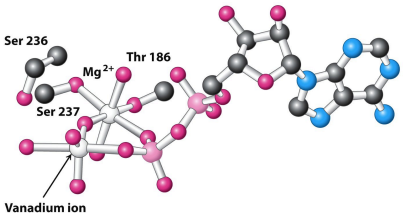
- An X-ray crystal structure of myosin II ATPase with ATP bound did not reveal how the γ-phosphate would be attacked by water.

An overlay with and without ATP



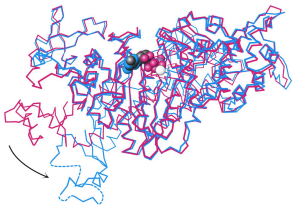
Myosin ATPase

- An X-ray crystal structure of myosin II ATPase with a transition state analogue for ATP revealed a mechanism
  - VO_4^{3-} + ADP was substituted for ATP.



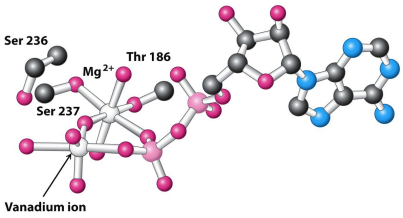
Myosin ATPase

- In order to stabilize the transition state the Myosin II ATPase must undergo a marked conformational change



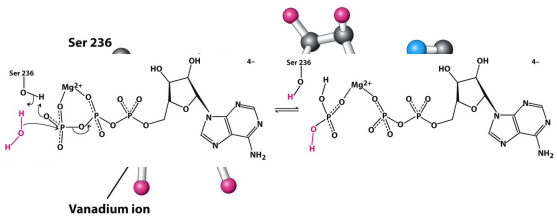
Myosin ATPase

- An X-ray crystal structure of myosin II ATPase with a transition state analogue for ATP revealed a mechanism
  - VO_4^{3-} + ADP was substituted for ATP.



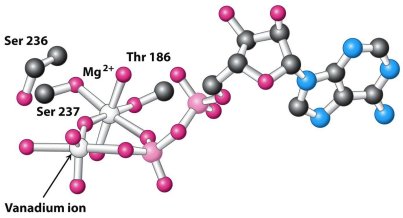
Myosin ATPase

- An X-ray crystal structure of myosin II ATPase with a transition state analogue for ATP revealed a mechanism
  - VO_4^{3-} + ADP was substituted for ATP.



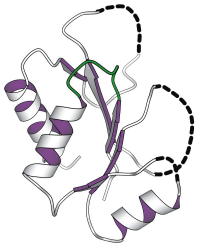
Myosin ATPase

- An X-ray crystal structure of myosin II ATPase with a transition state analogue for ATP revealed a mechanism
  - VO_4^{3-} + ADP was substituted for ATP.



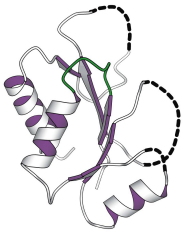
Myosin ATPase

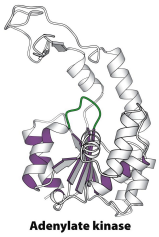
- Many NPTase share a similar binding domain for the nucleotide, and contains a peptide look called the "P-loop"



Myosin ATPase

- Many NPTase share a similar binding domain for the nucleotide, and contains a peptide look called the "P-loop"

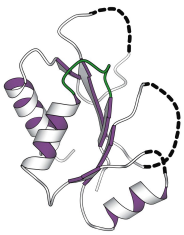




Adenylate kinase

Myosin ATPase

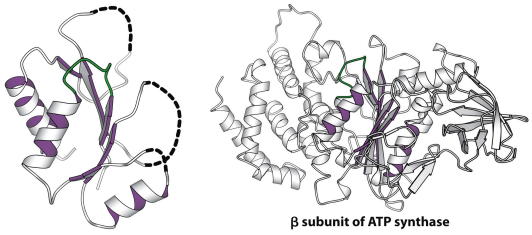
- Many NPTase share a similar binding domain for the nucleotide, and contains a peptide look called the "P-loop"



α subunit of transducin

Myosin ATPase

- Many NPTase share a similar binding domain for the nucleotide, and contains a peptide look called the "P-loop"



β subunit of ATP synthase

Next up

- Regulatory Strategies (Chapter 10)