Previous Class

• Detection of enzymatic intermediates: Protein tyrosine phosphatase mechanism

Today

Protein Kinase Catalytic Properties
Phosphorylation: key protein modification in biochemistry

- Hydrolysis of high energy ATP bond
- One in every Three proteins are phosphorylated
- Key regulatory functions: a number of key enzymes, signaling molecules, and oncogenes are controlled by phosphorylation/dephosphorylation, role in Signal Transduction
Protein Kinase Targets

**Serine/Threonine Kinases**

- Phosphoserine
- Phosphothreonine

**Tyrosine Kinases**

- Phosphotyrosine

**Dual Specific Kinases**
Protein Kinases Structural Features

Key to understanding protein kinase mechanism

No nucleophilic group on the enzyme

The nucleophilic attack is performed by hydroxyl oxygen of the substrate molecule

MgATP cofactor- source of phosphate, Mg neutralizes the triphosphates to allow localizing ATP in hydrophobic cleft of active site

Overall conserved fold creates environment for catalysis to occur

Phospho transfer is done by an in-line associative mechanism that involves a pentacoordinate phosphate intermediate
Protein Kinase Structural Features
• N-terminal lobe (beta sheets, Helix C) involved in ATP binding
• C-terminal lobe (helices, few beta strands) involved in Substrate binding (variable region) and Mg coordination
Protein Kinases Structural Features

N-terminal lobe:

**Glycine Loop** - GxGxxG – involved in localizing ATP in cleft

**Critical Lysine residue** – Invariant in every known protein kinase

Binds alpha and beta phosphates of ATP

Replacement of conserved Lys renders kinase “catalytically dead”

Km is unaffected (ATP still binds) but a 5700 fold decrease in kcat/Km is observed

**Conserved Glutamic acid** - Contacts side chain of invariant Lysine for positioning of ATP
Protein Kinases Structural Features

C-terminal lobe: Larger lobe

**Catalytic Loop** (HRD)– Contains an Aspartic acid that is presumed to act as a catalytic base to free up the hydroxyl oxygen on substrate for nucleophilic attack

**Activation Loop** (DFG…p(S/T/Y)….APE) – The Asp group interacts with Mg for positioning

Often contains a phosphorylation site that upon phosphorylation induces a conformational change on the loop that allows substrate to bind and also positions the catalytic Asp group of the catalytic loop

**Substrate binding region** – most variable part of kinases. Dictates specificity of kinases (Pro-directed, Basic, Acidic, Hydrophobic)
Activation Loop Phosphorylation

A  Inactive ERK

B  Active ERK
Tyr and Ser/Thr kinases have similar fold and mechanism.

Tyrosine Kinase Domain-with regulatory SH2 and SH3 domains

Serine/Threonine Kinase Domain
Phosphorylase Kinase
Ternary complex:
ATP analog (yellow) and Substrate Peptide (green)
Identification of an Essential Residue for Protein Kinase Catalysis

Group Specific Labeling to Identify Acidic Residues Involved in Catalysis:

Evidence for acidic groups being important:

Base catalysis was suspected due to nature of reaction

Carboxyl groups are frequently associated with metal binding sites on enzymes

Identification of an Essential Residue for Protein Kinase Catalysis

Experimental Rationale and Setup:

Ionized acid groups in hydrophobic active sites may be active

Dicyclohexylcarbodiimide (DCCD) is a hydrophobic compound that should localize to the active site and react with any ionized acidic groups present (give it a try)

Identify residue by trypsin digestion, HPLC purification and peptide sequencing

Then compete out the DCCD with MgATP cosubstrate to directly demonstrate involvement

First attempt: DCCD reacted but side reaction involving amines interfering with identification process
Blocking interfering Amino groups (Lys) with Acetic Anhydride

Acetic Anhydride + Amino Terminus of Protein or Peptide → Acetylated Amino Terminus

\[ \text{Acetyl} - \text{C} \cdot \text{NH} - \text{CH}_2 \cdot \text{C} \cdot \text{NH} \]
Labeling the DCCD with Glycine ethyl ester $[^{14}C]$

After protein kinase reacts with DCCD and Glycine ester the protein is incubated with trypsin that will cleave at the peptide bond at Arg and Lys sites.

The peptides are separated by HPLC using a C18 hydrophobic column and acetonitrile elution (polar peptides elute first)

The HPLC fractions that contain radioactivity contain the modified amino acid and is sequenced by Edman Degradation
Results and Conclusions

Two major radioactive peptides were observed after DCCD modification and reaction with Glycine ethyl ester [C14].

These peptides were sequenced and identified.

One amino acid was Glu 91 that is positioned in the N-terminal lobe that is now known to interact with the invariant critical Lys residue to position it for ATP binding. Mutation of Glu 91 results in a decrease in kcat/Km of 1000 fold.

The other amino acid was Asp 184 located in the C-terminal lobe. Although this paper postulates that it may be the catalytic base, X-ray structures show that it is the critical residue for chelating and positioning the Mg ion in the active site. Mutation of Asp 184 results in an inactive enzyme.
Conserved Mechanism:

Conformational change is the rate limiting step

Activation loop phosphorylation of enzyme induces active conformation

No phosphoenzyme intermediate formed

Mechanism involves an in-line phosphate transfer

Asp 166 acts as a catalytic base

Lys 72 forms ion pair with Glu 91 to coordinate alpha, beta phosphate groups of ATP

Mg interacts with beta and gamma phosphates of ATP

Mg is coordinated through contacts with Asp 184

Kinase-substrate-ATP complex is termed the ternary complex