Structural Basis of the Broad Variation of MK Feedback Inhibition

The MEV Pathway & MK

The **mevalonate (MEV) pathway** is an important metabolic pathway that produces steroid and isoprenoid precursors.[1-5]

Mevalonate kinase (MK) is a key enzyme in regulating the MEV pathway. MK phosphorylates mevalonate using ATP to produce 5-phosphomevalonate (Figure 1).[1-5]

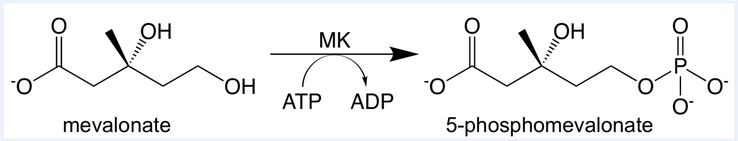

Figure 1: The MK-catalyzed reaction of the MEV pathway.

In many different organisms, competitive feedback inhibition regulates MK. The MK inhibitors have a broad variety in sizes, identities, and potencies but little is known about how MK is able to accommodate these wide variations. For example, how or why the same inhibitor may result in either weak or strong inhibition is unknown. In addition, some MK homologs are not affected by known inhibitors.[1]

Background

Research Questions:

- What is the structural basis of the broad variation in MK inhibition profiles?
  - How are inhibitors of different sizes able to bind to the same site?
  - Why do some inhibitors weakly affect MK while other inhibitors greatly affect MK, if at all?

Previous Findings:

*MK homologs that have crystal structures

MK homologs whose inhibition has been studied

- Feedback inhibited
  - **Rattus norvegicus* (RnMK)[4]
  - **Staphylococcus aureus* (SaMK)[6]
    - Weaker inhibition compared to the other MK homologs[5]
  - Human *Homo sapiens* (HsMK)[3]
  - *Saccharomyces cerevisiae* (ScMK)[1]
  - **Streptococcus pneumoniae* (SpMK)[3]
    - Inhibited by diphosphomevalonate (a different compound than the other inhibitors)
- Feedback resistant
  - **Methanosarcina mazei* (MmMK)[1]

MK homologs whose inhibition has not been studied yet (inhibition not conclusive)

- **Methanocaldococcus jannaschii* (MjMK)[2,7]
- **Leishmania major* (LmMK)[8]

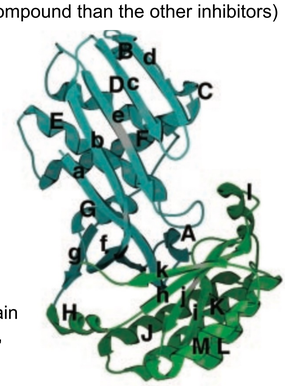

Figure 2: Secondary structures of MjMK: N-terminal domain (*cyan*), C-terminal domain (*green*), helices (*capital letters*), and the strands (*lowercase letters*).[2]

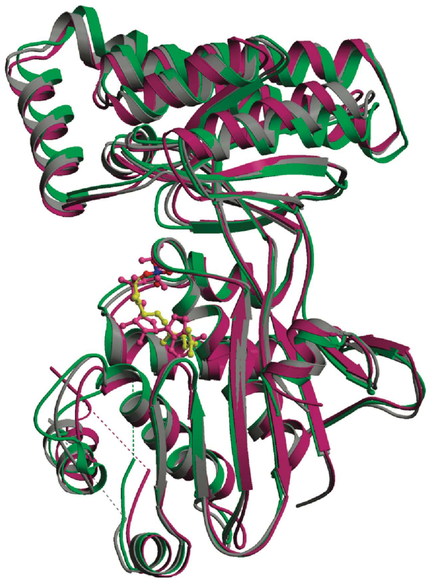

Figure 3: Overlay of three MK structures: apo HsMK (*green*), FSP bound to RnMK (*grey*), and ATP bound to RnMK (*magenta*). FSP (*yellow*) and ATP (*magenta*) shown in ball-and-stick. The C-terminal domain is shown at the top, while N-terminal domain is shown at the bottom. In all three structures, disordered regions are indicated by the dotted lines.[3]

Figure 4: The binding site of RnMK (*light blue*). ATP (*pink sticks*) and inhibitor FSP (*green sticks*) are superimposed to show competitive binding to the same site. The violet sphere represents Mg^{2+}. The inhibitor FSP is a FPP analog.[3,4]

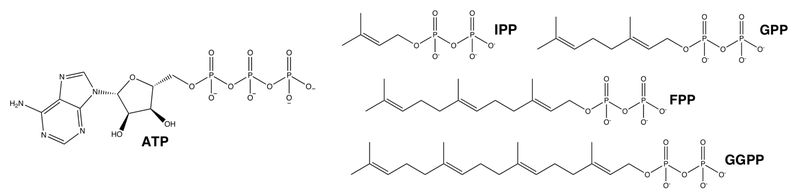

Figure 4: Structures of ATP and inhibitors which all bind to the same location. The inhibitors are geranylgeranyl pyrophosphate (GGPP), farnesyl pyrophosphate (FPP), geranyl pyrophosphate (GPP), and isopentyl pyrophosphate (IPP).

Objectives & Methods

Objectives:

- Obtain crystal structures of MjMK, SaMK, HsMK, ScMK bound to an inhibitor
- Perform parallel kinetic and inhibitions studies to complement the crystallographic work
- Use geranylgeranyl monophosphate (GGP), farnesyl monophosphate (FP), geranyl monophosphate (GP), and isopentyl monophosphate to perform inhibition studies with the MK homologs
- Use geranylgeraniol, farnesol, geraniol, isopentenol to perform inhibition studies with the MK homologs

Methods:

- Plasmid isolation & purification from *Escherichia coli*
- Transformation
- Bacterial cell culture & protein overexpression
- Cell lysis & protein purification
- Protein crystallization
- Kinetic & inhibition studies

Relevance & Importance

Large-scale connections of the MEV pathway & MK:

- Isoprenoids are a large and diverse class of natural products. Many are drugs used to treat various serious diseases, such as cancer and malaria.[1-5]
- Isoprenoid drugs can be made by expressing enzymes of the MEV pathway.[1-5]

The potential contribution of this work:

General

- Provides novel structural insight into enzyme regulation[1-5]
- Allows for the creation and use of modified enzymes which can be used for isoprenoid drug biosynthesis[1-5]
- May lead to the development of new antimicrobial drugs[1-5]

Specific

- Provides insight into why a diverse group of inhibitors can bind to the same site in a variety of MK homologs
  - Helps determine if the phosphate groups are a reason for why ATP and the inhibitors can bind to the same location
- Provides insight into why some MK inhibitors may be more potent than others
- Provides structural information on similarities and differences among MK homologs
- Helps to answer if and how MK changes its conformation to bind to ATP or an inhibitor

Conclusion

- The **mevalonate (MEV) pathway** is an important metabolic pathway that produces isoprenoid precursors. **Mevalonate kinase (MK)** is a key enzyme in regulating the pathway.[1-5]
- Competitive feedback inhibition regulates MK with respect to ATP in many different organisms.[1]
- Little is known as to how and why the diverse group of inhibitors can bind to the same site and bind with different potencies.
- Crystal structures of RnMK, MjMK, SaMK, MmMK, SpMK, and LmMK have been solved, including RnMK bound to ATP and inhibitor FSP.[2-7]
- The objectives of the study are to obtain structures of MjMK, SaMK, HsMK and ScMK bound to inhibitors, while characterizing them by performing kinetic and inhibition studies and determining if the phosphate groups are responsible for allowing the diverse group of inhibitors to bind at the same site.
- If this work is successful, then it may provide insight into enzyme regulation and allow the creation of new antimicrobial drugs and drugs used to treat various serious illnesses.[1-5]

References

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