**“A likely story” - Enzyme Inhibition Case Study**

“I’m telling you I didn’t do it!” cried Mrs. Bleu. “He just fell on my knife!” The detective smirked, turning away to hold back a chuckle.

“I don’t know why” she continued, “he ju—, he… he just sort of turned to me gasping and fell forward... I was just making dinner, chopping broccoli...” Mrs. Bleu trailed off and looked longingly at the floor of the interview room. They had been there for hours. “He was just standing at the end of the kitchen drinking his coffee and looking through the mail when it happened, but it was an accident. Why would I kill my husband?”

 “We don’t know why”, the detective said calmly, “we’re hoping you’ll tell us. Your husband was found on the kitchen floor in a pool of blood...*his* blood. He had a knife-wound, a wound from a kitchen knife that was lying on the floor just feet away covered in his blood. His blood…and *your* fingerprints! Moreover, you told the 911 operator that *you* stabbed him—”

“Accidentally!” she gasped.

“Ok…ok, you admitted to accidentally stabbing him…but do you really expect us to believe that he just *fell* onto your knife? Do you know how ridiculous that sounds?”

“I don’t care if it sounds ridiculous! It’s what happened!” she said, exasperated. “Look… I need to know what happened to my husband. One minute he was fine, and the next he was gasping! He had a heart attack I think, er--, I don’ t know, but he was MY husband. Why won’t you tell me?”

The detective looked annoyed. “We haven’t heard back yet from the coroner Mrs. Bleu… but this is an active homicide investigation. We will share that information with you when we get it. Believe me, when we get confirmation of the cause of death, we will be talking again…”

He stepped out into the hallway. He had heard some good ones, but “he fell on my knife”? *Really*? At that moment, the coroner stepped off the elevator and into the hallway… she was out of breath and appeared to be sweating.

“Frank! Frank, listen! I just finished the autopsy. It missed!”

“What? What missed? Was it a homicide?”

“No! I mean, maybe. Er—I don’t know for sure yet, we need the Tox. results.”

“Toxicology results? …for a stabbing? And what do you mean *it missed*?”

“That’s just it, the knife! It missed the brachial artery! It was only muscle damage, mostly venous bleeding.Seeping blood comes out slow. It was halfway coagulated before he died and it wasn’t even that deep.”

“What? Will you just calm down and start talking in plain English! What are you trying to say?”

“It was a flesh wound…plenty of blood, but not enough to kill a person. Without arterial damage it would take a long time to bleed out from this wound. But there is more to it…this guy died shortly after he was stabbed, but it wasn’t from the knife, that much is for sure!”

The detective cursed under his breath…it was going to be a long day. “Okay, so the guy didn’t die from the knife wound, but he is dead, so from *what*?!!”

Two weeks have passed and you have been brought in to help investigate this case. You have heard the interrogation tapes and been briefed by both the lead detective and the coroner. The coroner has ruled the cause of death of Dr. John Bleu a homicide, but by poisoning, rather than because of the knife-wound inflicted by Mrs. Bleu, presumably now by accident. Small quantities of an unknown substance, hereafter referred to as “Compound X”, were discovered in the decaf-coffee Dr. Bleu was drinking when he died. Dr. Bleu had brought the coffee home with him from work as he did everyday according to co-workers and his wife. His wife is still under suspicion, but it has not escaped the notice of the investigators that Dr. Bleu leads a large research group studying potentially lethal enzyme inhibitors for the drug company *Hinesbiopharma*.

The company is working with the U.S. government in a counter-terrorism effort to study the effects of chemical weapons so that potential treatments can be created in advance. Three scientists who Dr. Bleu supervised are running independent projects studying different inhibitors toward the same enzyme, referred to in company documents as XYZase. XYZase is critical to human metabolism and so development of chemical inhibitors of this enzyme by terrorist groups is viewed as a real and present threat by counter-terrorism experts. The three scientists are **Dr. Greene**, **Dr. Gray**,and **Dr. Wight**. According to the company policies, only Dr. Bleu and the single scientist assigned to each project have access to the highly valuable but extremely lethal compounds each one is studying.

To complete this case, you will be given enzyme kinetic data, and you will need to use Microsoft EXCEL (or another graphing program) to graph the data in order to determine the kinetic parameters and type of inhibition. A tutorial to guide you through using MS EXCEL is also provided.

You decide to…

**Run kinetics experiments on XYZase without inhibitors and with Compound X**

**Results:** The compound found in the coffee (Compound X) has yet to be identified, but using the small amount extracted, you have discovered that it is an inhibitor of XYZase. Given this revelation, it seems clear that this compound is the most likely the murder weapon. Shown below is the data you have gathered. Note: [S] is given in nM; other values represent observed V0 values given in units of µmol product formed per minute.

|  |  |  |
| --- | --- | --- |
| **[S]** | **V0(No inhibitor)** | **V0 (Murder Weapon - Cmpd. X)** |
| 50 | 0.14 | 0.040 |
| 75 | 0.19 | 0.050 |
| 150 | 0.32 | 0.080 |
| 400 | 0.47 | 0.14 |

1. From the limited data you have gathered, does it appear that XYZase follows Michaelis-Menten kinetics?
	1. Yes
	2. No
2. **Calculation, fill in the blank:** What is the KM for XYZase within two significant figures? \_\_\_\_\_nM
3. **Calculation, fill in the blank**: Assuming that the experiments performed in the absence of inhibitors were conducted by adding 5µL of a 2mg/mL enzyme stock solution to an assay mixture with a 1mL total volume, and taking into account that XYZase is a monomeric enzyme with a molecular mass of 45,000 Daltons, what is the kcat\* of XYZase given in s-1 (give the answer in to two significant figures)? \_\_\_\_\_\_\_s-1

\*Flip to the end of this case study to see a sample calculation for kcat.

Given that Dr. Bleu appears to have been killed by an inhibitor to XYZase, there is now sufficient cause to search the labs of Drs. Greene, Gray, and Wight so that you can recover and test samples of the compounds they are each developing. This discovery has opened up a new investigation option! You decide to…

**Search the labs and run kinetics experiments on suspicious samples**

**Results:** By gathering samples from the labs of Drs. Greene, Gray and Wight you hope to discover which of these people may have had access to what is now clearly the murder weapon, Compound X. Shown below is the data you have gathered. Note: [S] is given in nM; other values represent observed V0 values given in units of µmol product formed per minute.

|  |  |  |  |
| --- | --- | --- | --- |
| **[S]** | **V0 (Sample 1)** | **V0 (Sample 2)** | **V0 (Sample 3)**  |
| 50 | 0.080 | 0.067 | 0.055 |
| 75 | 0.11 | 0.080 | 0.063 |
| 150 | 0.19 | 0.14 | 0.086 |
| 400 | 0.36 | 0.24 | 0.10 |

1. **Multiple Choice:** Because you do not know with certainty the amounts of each inhibitor in the solution (for example, the amount of the murder weapon you are adding to the assay), you cannot directly compare the magnitude of inhibition between the murder weapon and the gathered samples. How, then, can these samples be distinguished and compared by using enzyme kinetics?
2. By the magnitude of the inhibition
3. By the shape of the Michaelis-Menten curve
4. The type of enzyme inhibitor can be distinguished by kinetics
5. The inhibitors cannot be distinguished using kinetics under any circumstances
6. The inhibitors cannot be distinguished using the data given
7. **Matching:** Based on the information you have gathered, and your answers to previous questions, match the sample to the observed type of enzyme inhibition. [Hint: You must graph the data and examine how each inhibitor affects the observed kinetic parameters.]

 Sample 1 Noncompetitive

 Sample 2 Uncompetitive

 Sample 3 Competitive

You notice, however, that the samples you collected from the labs are now labeled 1, 2 and 3. You ask your lab assistants which samples correspond to which labs, but they mixed up the samples (somebody didn’t label the tubes when running the assays!). This is a disaster! Now, in addition to determining which sample (if any) matches the murder weapon, you are going to have to testify in court as a biochemist to convince a jury that the guilty sample (1, 2, or 3) belongs to the guilty person (Drs. Greene, Wight, or Gray) based on what you know about enzyme kinetics! Are you up to the challenge? Who killed Dr. Bleu?

The good news is that you may now go back to *Hinesbiopharma* and interview the three scientists about the molecular mechanism of the inhibitor each is working with.

**You begin by interviewing…**

**Dr. Gray**

**Results**: Dr. Gray is studying the effects of a transition-state analog (Tymoczko 3E pgs. 104-105 & 138). This molecule mimics the geometry of the theoretical transition state in the XYZase reaction mechanism. Dr. Gray has solved crystal structures that have confirmed that the inhibitor binds non-covalently in the active site as expected.

1. **Multiple Choice:** What type of inhibitor is being studied by Dr. Gray?
2. Competitive
3. Uncompetitive
4. Irreversible
5. Noncompetitive

**Next you interview…**

**Dr. Greene**

**Results:** Dr. Greene is studying a compound that binds non-covalently to an allosteric site (Tymoczko 3E pgs. 118-122) on the protein approximately 20Å away from the active site. She has solved crystal structures, which reveal that this allosteric site is blocked in the absence of substrate. When the enzyme binds the substrate, a shift in a helix connecting the active site and the allosteric site, clears the site and allows the inhibitor to bind. Binding studies have confirmed predictions made by the crystal structures: in the absence of substrate, the inhibitor fails to bind to the enzyme.

1. **Multiple Choice:** What type of inhibitor is being studied by Dr. Greene?
2. Competitive
3. Uncompetitive
4. Irreversible
5. Noncompetitive

**Finally, you interview…**

**Dr. Wight**

**Results**: Dr. Wight works on an inhibitor of XYZase that acts by binding to a lysine residue in the enzyme active site, to form a Schiff-base, which is a covalent linkage. This lysine is critical to function, normally acting as an acid in the enzyme mechanism. Stability studies have confirmed that under physiological conditions, the inhibitor- Schiff-base linkage formed in the enzyme active site is extremely stable, with a half-life of more than 24 hours.

1. **Multiple Choice:** Based on the information that you have about the inhibitor that Dr. Wight is studying, what type of inhibitor do you think he is working with?
2. Competitive
3. Uncompetitive
4. Noncompetitive
5. Irreversible
6. **Multiple Choice:** If you were to analyze Dr. Wight’s inhibitor using enzyme kinetics, you would find that the numbers look very much like those that you would get if you had a certain type of reversible inhibitor. Irreversible inhibitors render the enzyme molecules they bind, completely unable to perform catalysis. The effect is similar to decreasing the concentration of the enzyme in the enzyme assay. How does an irreversible inhibitor affect KM and Vmax? What type of reversible inhibitor does an irreversible inhibitor resemble in this manner?
	1. Vmax unchanged, KM increased; looks like a competitive inhibitor
	2. Vmax decreased, KM decreased; looks like an uncompetitive inhibitor
	3. Vmax decreased, KM unchanged; looks like a noncompetitive inhibitor

**Final Assessment questions:**

1. **Matching:** Based on the information you have gathered, and your answers to previous questions, match the samples to their respective scientists. [Hint: review the information from interviews and answers to previously answered questions.]

 Sample 1 Dr. Greene

 Sample 2 Dr. Gray

 Sample 3 Dr. Wight

1. **Multiple Choice:** What type of inhibitor was compound X?
2. Competitive
3. Uncompetitive
4. Noncompetitive
5. Irreversible
6. **Multiple Choice:** Based on their kinetics, which sample matches compound X?
7. Sample 1
8. Sample 2
9. Sample 3
10. **Multiple Choice:** Based on your answers to the previous questions, who is the murderer?
11. Dr. Gray
12. Dr. Wight
13. Dr. Greene

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**\*Sample kcat calculation:**

In an enzyme assay, you determine the KM for your enzyme to be 0.50 mM. You measure the maximum rate of the reaction to be 0.050 mmoles/second. Calculate kcat for this reaction if the above experiment was conducted with a 1mL reaction volume at an enzyme concentration of 100 nM.

kcat = Vmax/Etotal

[E] = 100 nM

Etotal = [E] x volume

Etotal = (100 nM)(1mL)

Etotal = (1 x 10-7 M/L)(1 x 10-3 L)

Etotal = 1 x 10-10 moles

 Vmax = 0.05 mmoles/second

kcat = Vmax/Etotal

kcat = (0.05 mmoles/second) / (1 x 10-10 moles)

kcat = (5 x 10-5 moles/second) / (1 x 10-10 moles)

kcat = 500,000 s-1

**Note:** In this problem knowing the value of KM was irrelevant…this is true because the value of Vmax was given. If in another problem we were instead given the initial velocity (V0) at some concentration of substrate, then we could use the Michaelis-Menten equation, and the value of KM, to first calculate Vmax!

**Also Note:** When V0 is given in units of **molarity** per unit time, then kcat = Vmax/[Et] where [Et] is the **concentration** of the enzyme in the assay; however, when Vmax is given in **moles** per unit time, then kcat = Vmax/Et, where Et is the number of **moles** of Et in the assay.