

Advanced Multiple Choice Questions

Xanthine Oxidase Enzyme Inhibition with Allopurinol

1. Which statement best explains the biological importance of xanthine oxidase?

- A) It catalyzes lipid digestion and prevents oxidative stress in tissues.
- B) It participates in purine catabolism and generates reactive oxygen species during oxidation reactions.
- C) It converts amino acids into glucose during starvation conditions.
- D) It hydrolyzes proteins into peptides within lysosomes.

2. The conversion of hypoxanthine into xanthine by xanthine oxidase requires the presence of:

- A) ATP and NADH only
- B) Oxygen and water molecules
- C) Carbon dioxide and phosphate buffer
- D) Glucose and magnesium ions

3. Why can xanthine oxidase contribute to cellular oxidative damage?

- A) Because it consumes uric acid from the bloodstream.
- B) Because it produces hydrogen peroxide and oxygen-derived free radicals during catalysis.
- C) Because it directly destroys DNA by hydrolysis.
- D) Because it decreases oxygen concentration in mitochondria.

4. Hyperuricemia associated with gout mainly results from:

- A) Excessive degradation of fatty acids inside the liver
- B) Increased uric acid accumulation and deposition in joints
- C) Deficiency of phosphate buffer in plasma
- D) Increased glucose oxidation inside cells

5. Which statement best describes the therapeutic role of allopurinol?

- A) It increases urinary excretion of glucose by the kidneys.
- B) It activates xanthine oxidase to accelerate purine metabolism.
- C) It inhibits xanthine oxidase and reduces uric acid biosynthesis.
- D) It directly dissolves uric acid crystals already deposited in joints.

6. In the assay procedure, why was phosphate buffer used in the reaction mixture?

- A) To supply ATP required for enzyme activity
- B) To maintain an appropriate pH environment for the enzymatic reaction
- C) To convert xanthine into hypoxanthine
- D) To chemically inhibit allopurinol activity

7. The absorbance was measured specifically at 295 nm because this wavelength is associated with:

- A) Detection of the reaction products formed during xanthine oxidation
- B) Measurement of dissolved oxygen concentration only
- C) Maximum absorption of phosphate buffer alone
- D) Visualization of protein precipitation inside the wells

8. What is the main purpose of including a blank well in this experiment?

- A) To increase enzyme concentration during incubation
- B) To determine the spontaneous oxidation rate of allopurinol
- C) To correct absorbance readings by excluding background absorbance
- D) To calculate the molecular weight of xanthine oxidase

9. Why was the reaction mixture preincubated before addition of the enzyme?

- A) To denature allopurinol before catalysis begins
- B) To allow temperature equilibration and proper interaction of reaction components
- C) To convert uric acid back into xanthine
- D) To completely consume the substrate before incubation

10. Which factor directly initiates the enzymatic reaction in this assay?

- A) Addition of phosphate buffer
- B) Addition of xanthine substrate
- C) Addition of xanthine oxidase enzyme
- D) Increasing incubation temperature to 50°C

11. In the inhibition equation, the value A_{max} represents:

- A) Absorbance of the blank without substrate
- B) Enzyme activity measured in the presence of inhibitor
- C) Maximum theoretical absorbance of the plate reader

D) Absorbance of phosphate buffer at zero time

12. Why does increasing allopurinol concentration generally increase the percentage inhibition?

- A) Because higher inhibitor concentration blocks a greater proportion of enzyme activity
- B) Because allopurinol converts uric acid into hypoxanthine
- C) Because phosphate buffer becomes more acidic at higher concentrations
- D) Because the substrate concentration increases proportionally

13. The final concentration of allopurinol inside the well becomes lower than the stock concentration because:

- A) The inhibitor is degraded by phosphate buffer immediately
- B) The inhibitor undergoes oxidation during incubation
- C) The inhibitor becomes diluted after mixing with other reaction components
- D) The inhibitor binds irreversibly to the plastic surface of the plate

14. Which statement best describes IC₅₀?

- A) The concentration required to completely activate the enzyme
- B) The concentration of inhibitor that produces 50% maximal inhibition
- C) The concentration of substrate needed to double absorbance
- D) The concentration at which uric acid becomes undetectable

15. Why is a logarithmic equation used during IC₅₀ determination?

- A) Because inhibition responses commonly show logarithmic relationships with inhibitor concentration
- B) Because absorbance values cannot be analyzed mathematically
- C) Because xanthine oxidase only functions under logarithmic conditions
- D) Because phosphate buffer follows exponential kinetics

16. In the provided example, the maximum inhibition observed was 97%. Therefore, the IC₅₀ corresponded to:

- A) 24.25% inhibition
- B) 48.5% inhibition
- C) 50% absorbance increase
- D) 97% substrate consumption

17. Which statement correctly explains the importance of reactive oxygen species mentioned in the experiment?

- A) They stabilize DNA and protect cells from mutations.
- B) They enhance protein synthesis during oxidative metabolism.
- C) They may damage DNA, lipids, and proteins, increasing disease risk.
- D) They prevent accumulation of uric acid in plasma.

18. Why are gout patients commonly treated with xanthine oxidase inhibitors instead of enzyme activators?

- A) Because inhibition decreases uric acid formation and reduces crystal deposition in joints
- B) Because activation promotes antioxidant synthesis in cartilage
- C) Because enzyme activation prevents purine metabolism completely
- D) Because inhibition increases production of hydrogen peroxide

19. During graph preparation in Excel, the inhibitory activity was plotted against:

- A) Enzyme molecular weight
- B) Incubation temperature
- C) Final inhibitor concentration
- D) Phosphate buffer volume

20. Which experimental condition would most likely produce inaccurate inhibition results?

- A) Correct blank subtraction before calculations
- B) Maintaining incubation temperature at 25°C
- C) Failure to subtract background absorbance from the blank well
- D) Using the recommended xanthine concentration in all wells

Answer Key

1. B

2. B

3. B

4. B

5. C

6. B

7. A

8. C

9. B

10. C

11. B

12. A

13. C

14. B

15. A

16. B

17. C

18. A

19. C

20. C