# Enzymes 2

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# Quiz -2

Identify each description as an inhibitor that is

1) competitive or 2) noncompetitive.

A. Increasing substrate reverses inhibition.
B. Binds to enzyme surface, but not to the active site.
C. Structure is similar to substrate.
D. Inhibition is not reversed by adding more substrate.

# solution

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# MEASUREMENT OF ENZYME ACTIVITY

- The enzyme activity can be defined as the number of moles of substrate converted per unit time.

- Enzyme activity = moles of substrate converted per unit time
- = rate × reaction volume.

- The SI unit is the katal, 1 katal = 1 mol $s^{-1}$
- A more practical and commonly used value is 1 enzyme unit (U) = 1 µmol $min^{-1}$

# TYPES OF ENZYME ASSAYS

## CONTINUOUS ASSAYS

- With continuous assays, one can measure the linearity of the assay which can be used to conduct a fixed-timed assay
- A few methods are spectrophotoometric, fluorometric, calorimetric and chemi-luminescent.

## DISCONTINUOUS ASSAYS

- Discontinuous assays are when samples are taken from an enzyme reaction at intervals and the amount of product production or substrate consumption is measured in these samples.
- The discontinuous assays are radiometric and chromatographic.

# MEASUREMENT OF ENZYMATIC ACTIVITY BY SPECTROSCOPY

- Spectrophotometry is the measureable analysis technique using the electromagnetic spectra. It deals with the ranges of wavelengths such as near ultraviolet, near infrared and visible light

- Activity of the enzyme, the following spectroscopic techniques are used: Fluorescence spectroscopy, UV/VIS Spectroscopy, Spectrophotometric Assays, and Infrared spectroscopy.

# FLUORESCENCE SPECTROSCOPY

- Fluorescence spectroscopy reveals the existence of ES complexes and what they are made of.

- A compound is exposed to UV-light which excites certain molecules and causes them to emit light at a lower wavelength, which is in the visible light range.

- The fluorescence of the substrate is measured and compared to the fluorescence of the product, and in the difference of the two measurements, enzymatic activity is measured

# INFRARED SPECTROSCOPY

- In enzyme-substrate complexes, there are well-organized binding modes, which is quantifiable using infrared methods.

- In analyzing infrared data, it is possible to identify binding modes and heterogeneity of ES complexes.

# ULTRAVIOLET-VISIBLE SPECTROSCOPY

- It is a commonly used spectrophotometric assay that examines photons in the UV-visible region.

- It is mainly used to determine the amount of a highly-conjugated organic compound or enzyme contained in a specific solution.

- It is complimentary to fluorescence spectroscopy, as fluorescence spectroscopy deals with transitions from the excited state to the ground state, ultraviolet-visible spectroscopy deals with transitions from the ground state to the excited state.

- The device, spectrophotometer, measures the transmittance of light through a sample

The equation used to calculate the transmittance is

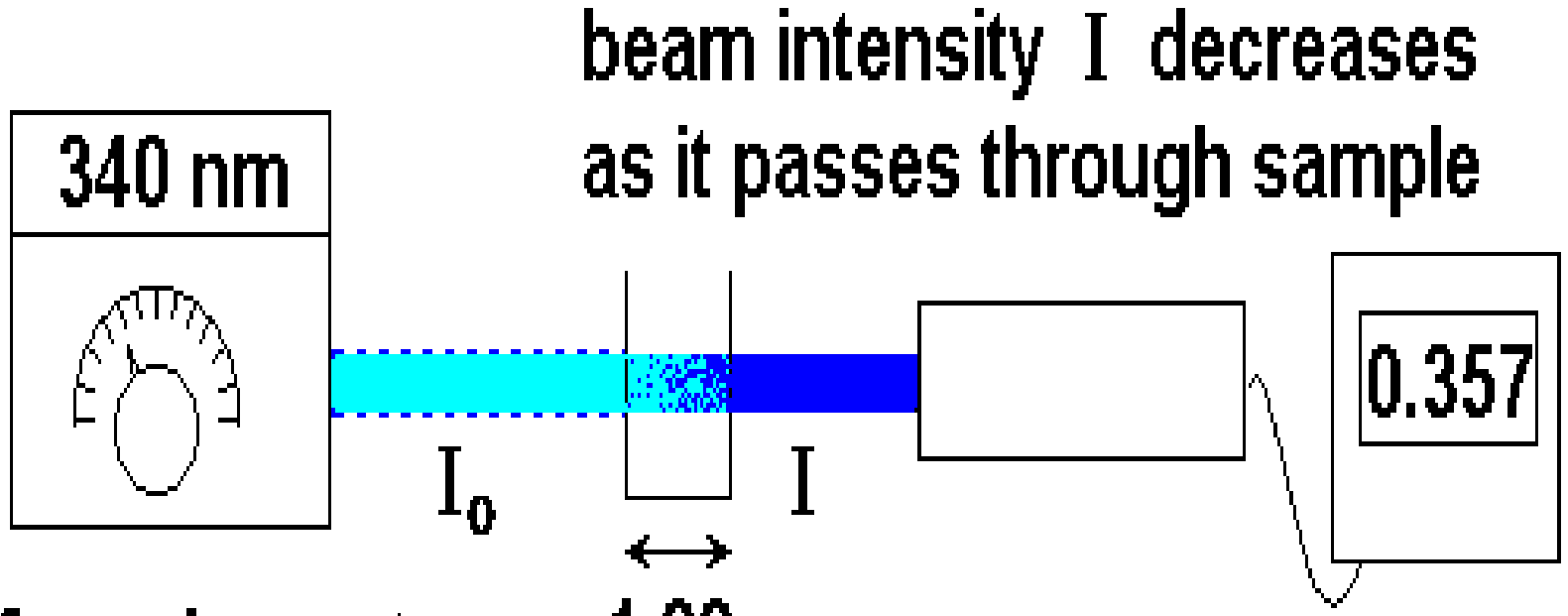
$$A=-\log(\%T)$$

Here, A= absorbance

T= I/Io

I=the intensity of light passing through the sample

Io=the initial intensity of the light before it is transmitted through the sample




Monochromator:
light source
with selectable
wavelength

Sample
in solution

Detector and
readout or
printout

# RADIOMETRIC ASSAY

- It measures the incorporation of radioactivity into substrates or its release from substrates.
- The radioactive isotopes most frequently used in these assays are $^{14}C$, $^{32}P$, $^{35}S$ and $^{125}I$.
- These assays are both extremely sensitive and specific.
- They are often the only way of measuring a specific reaction in crude extracts (the complex mixtures of enzymes produced when you lyse cells).

# CHROMATOGRAPHIC ASSAY

- It measures product formation by separating the reaction mixture into its components by chromatography.

- This is usually done by high-performance liquid chromatography (HPLC), but can also use the simpler technique of thin layer chromatography.

- Although this approach can need a lot of material, its sensitivity can be increased by labelling the substrates/products with a radioactive or fluorescent tag, by switching protocols to improved chromatographic instruments (e.g. ultra-high pressure liquid chromatography) that operate at pump pressure a few-fold higher than HPLC instruments.

# Specificity of Enzymes

- 1. **Absolute specificity** - the enzyme will catalyze only one reaction.
- 2. **Group specificity** - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- 3. **Linkage specificity** - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
- 4. **Stereochemical specificity** - the enzyme will act on a particular steric or optical isomer.

# Naming and Classification

Except for some of the originally studied enzymes such as pepsin, rennin, and trypsin, most enzyme names end in "ase". The International Union of Biochemistry (I.U.B.) initiated standards of enzyme nomenclature which recommend that enzyme names indicate both the substrate acted upon and the type of reaction catalyzed. Under this system, the enzyme uricase is called urate: O2 oxidoreductase, while the enzyme glutamic oxaloacetic transaminase (GOT) is called L-aspartate: 2-oxoglutarate aminotransferase.

# Enzymes can be classified by the kind of chemical reaction catalyzed.

- I. Addition or removal of water
- A. Hydrolases - these include esterases, carbohydrases, nucleases, deaminases,
- amidases, and proteases
- B. Hydrases such as fumarase, enolase, aconitase and carbonic anhydrase
- II. Transfer of electrons
- A. Oxidases
- B. Dehydrogenases

- III. Transfer of a radical
- A. Transglycosidases - of monosaccharides
- B. Transphosphorylases and phosphomutases - of a phosphate group
- C. Transaminases - of amino group
- D. Transmethylases - of a methyl group
- E. Transacetylases - of an acetyl group

- IV. Splitting or forming a C-C bond
- A. Desmolases
- V. Changing geometry or structure of a molecule
- A. Isomerases
- VI. Joining two molecules through hydrolysis of pyrophosphate bond in ATP or other
- tri-phosphate
- A. Ligases

**Enzymes Are Classified into six functional Classes (EC number Classification) by the International Union of Biochemists (I.U.B.). on the Basis of the Types of Reactions That They Catalyze**

- **EC 1. Oxidoreductases**
- **EC 2. Transferases**
- **EC 3. Hydrolases**
- **EC 4. Lyases**
- **EC 5. Isomerases**
- **EC 6. Ligases**

# Principle of the international classification

Each enzyme has **classification number**

consisting of four digits:

Example, **EC:** (**2.7.1.1**)
**HEXOKINASE**

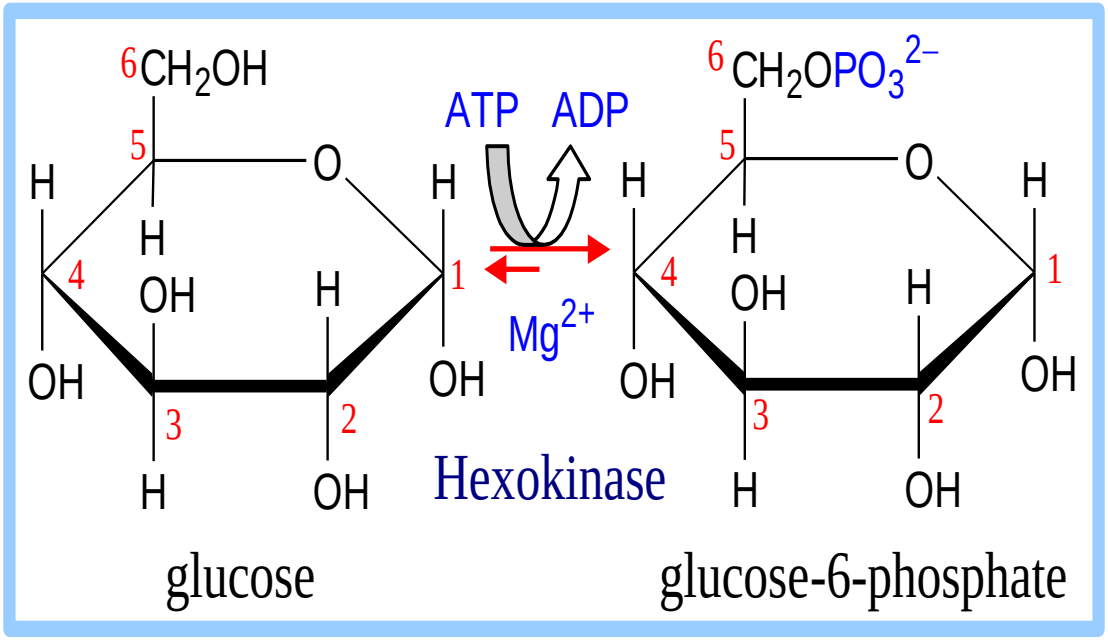
- **EC: (2.7.1.1) these components indicate the following groups of enzymes:**

- **2. IS CLASS (TRANSFERASE)**

- **7. IS SUBCLASS (TRANSFER OF PHOSPHATE)**

- **1. IS SUB-SUB CLASS (ALCOHOL IS PHOSPHATE ACCEPTOR)**
- **1. SPECIFIC NAME**

  **ATP,D-HEXOSE-6-PHOSPHOTRANSFERASE (Hexokinase)**




**1. Hexokinase** catalyzes:

**Glucose + ATP → glucose-6-P + ADP**