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BioChemistry | FINAL 13

Enzymes pt.5

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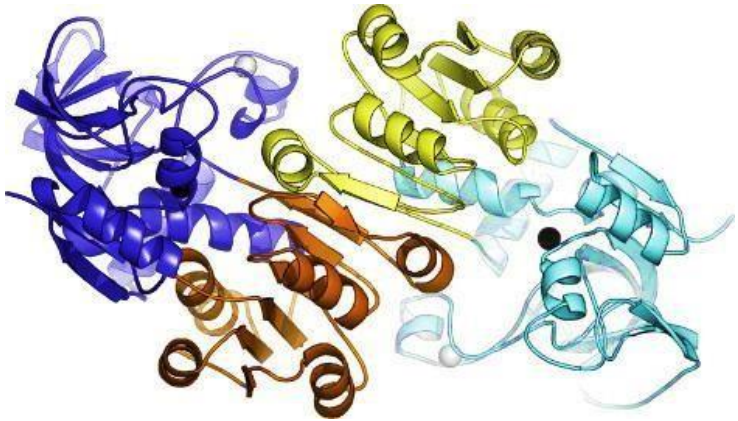
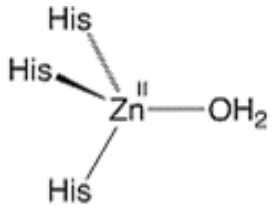
Metalloenzymes

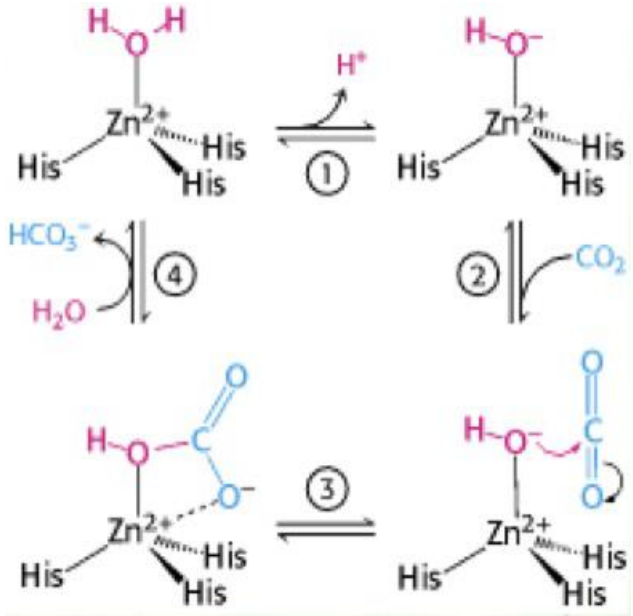
- Metal ions are usually incorporated during synthesis & removal of the metal causes denaturation
- These metal ions may contribute either to the structure or the catalytic mechanism
- Liver alcohol dehydrogenase (dimer); 2 Zn^{+2} in each monomer; one for structural maintenance (joins the two subunits), the other is catalytic
- Carbonic anhydrase; A zinc atom is essentially always bound to four or more groups





[NNN]Zn^{II}–OH_2

Carbonic Anhydrase



Kinetics of enzymatic reactions

A QUICK RECAP FROM THE PREVIOUS LECTURE ::

- ✓ **kinetics of enzymatic reactions** :: It is the science that deals with biochemically catalysed reaction rates.
- ✓ **Rate** should be similar in between reactants and products. We define the rate as :: the rate constant for that specific reaction multiplied by the reactants. $v = k(A)^{n1}(B)^{n2}(C)^{n3}$
- ✓ **The enzymatic reaction rate** has a limit, which is controlled by the availability of the active sites on the enzyme. This limitation gives us an important parameter in enzyme kinetics called Vmax.
- ✓ **Vmax** represents the maximum velocity that can be achieved by an enzyme-catalyzed reaction when all active sites of the enzyme molecules are fully saturated with substrate. It reflects the maximum number of substrate molecules that the enzyme can convert into product per unit time.

A QUICK RECAP FROM THE PREVIOUS LECTURE ::

Michaelis and Menten
have studied a large number of enzymes and observed that enzymes show two main types of kinetic behavior:

1. Hyperbolic behavior ~>seen with simple, Michaelis-Menten type enzymes.
2. Sigmoidal behavior ~>seen with allosteric enzymes.

To understand the hyperbolic type, scientists derived a mathematical expression that explains how these enzymes function. After going through the derivation, the final equation you must know, memorize, and be able to apply and manipulate in solving problems is the Michaelis-Menten equation:

$$v = \frac{Vmax\ S}{K_m + S}$$

From this formula, we identify an important parameter called the Michaelis constant (Km).

- Km is a combination of constants grouped together from the derivation.

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

- Definition: Km is the substrate concentration at which the reaction velocity equals half of Vmax.
- Meaning: Km indicates the affinity of the enzyme for its substrate.
- A low Km means high affinity (the enzyme reaches half Vmax at a low [S]).
- A high Km means low affinity (it requires more substrate to reach half Vmax).
- Application on the plot: On the hyperbolic curve (υ vs. [S]), Km corresponds to the [S] value where

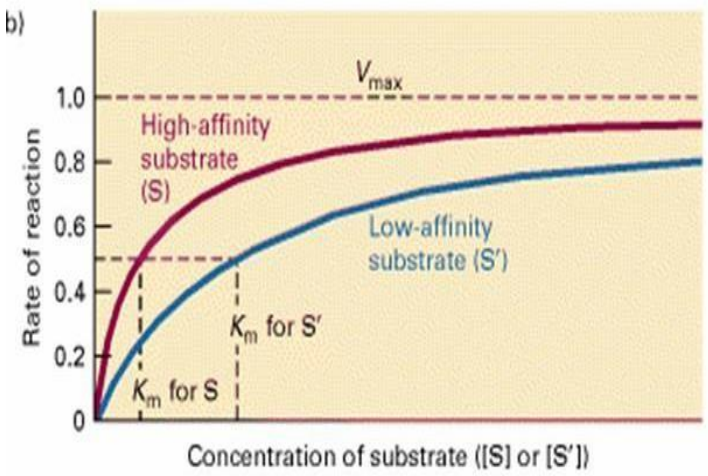
$$K_m = [S], \text{ when } V_0 = \frac{1}{2}V_{max}$$

A QUICK RECAP FROM THE PREVIOUS LECTURE ::

We have mentioned that if we are dealing with a single reaction that produces one product, then there should be one reaction rate value for that process.

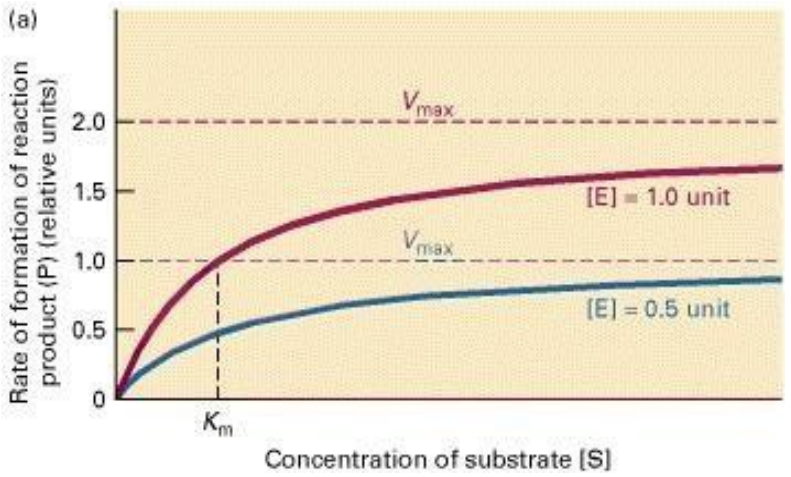
However, if there is more than one substrate, the enzyme does not bind them all with the same affinity. Accordingly, we will obtain two plots representing this type of reaction. These plots will eventually merge at the same Vmax value, but they differ in their Km values.

In some cases, when catalyzing the reaction with different substrates, we may also obtain two plots with different Vmax values.
For example, hexokinases and glucokinases can catalyze the phosphorylation of glucose, and sometimes of other sugars such as fructose and mannose. By any means, the rate of production of glucose-6-phosphate cannot be identical to the rate of production of mannose-6-phosphate or fructose-6-phosphate. Each substrate has its own Vmax value. They may share the same Km for ATP, but they have different Km values for glucose, fructose, and mannose.

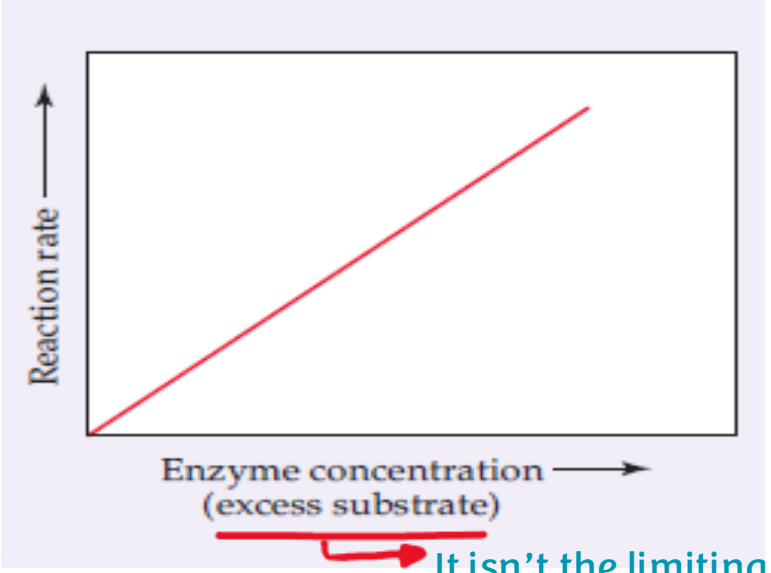


The limiting factor of an enzyme-catalyzed reaction is the number of active sites available. If you increase the number of active sites, the reaction rate will also increase ; as long as there is no other limiting factor, such as substrate concentration.

So, when the substrate concentration is in excess, the availability of active sites becomes the limiting factor. Increasing the number of enzyme molecules (and thus the number of active sites) increases the reaction rate proportionally.



A classical biochemical question is: if we perform an experiment with a certain enzyme concentration, and then repeat the experiment with a different enzyme concentration, what happens to the reaction rate? The answer is that the new rate is directly proportional to the enzyme concentration – it will be multiplied or divided according to the change in enzyme amount.



It isn't the limiting factor

1. Limiting factor of enzyme velocity

· The limiting factor for the velocity of an enzyme-catalyzed reaction is the number of active sites available.

2. Vmax and enzyme concentration

· Suppose we have two reactions:
· Reaction 1: Vmax = 10 → catalyzes 10 reactions per second.
· Reaction 2: Vmax = 20 → catalyzes 20 reactions per second.
· Which enzyme is more efficient? We cannot answer without considering enzyme concentration.
· For example, if the first enzyme has 1 unit of enzyme and the second has 5 units, the first enzyme is more efficient per unit enzyme, because the second enzyme's higher Vmax is due to higher concentration.
· Conclusion: Vmax depends on enzyme concentration. Increasing the enzyme concentration increases Vmax.

3. Normalization: Kcat

· To compare enzymes fairly, we normalize Vmax by enzyme concentration.
· This gives the catalytic constant Kcat, which represents the number of substrate molecules converted per enzyme molecule per unit time.

$k_{cat} = V_{max} / [E]_T$

Where $[E]_T$ is the total enzyme concentration.
· Kcat allows us to compare enzyme efficiency independent of enzyme concentration.

4. Relation to enzyme-substrate reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

· Consider the reaction:

· Here:

· k_1 = rate constant for formation of ES complex
· k_{-1} = rate constant for dissociation of ES
· k_2 = rate constant for conversion of ES to product (same as Kcat)

· At Vmax, all enzyme is in the ES complex:

$$ES] = [E]_T$$

· Therefore:

· To calculate Kcat:

$k_{cat} = V_{max} / [E]_T$

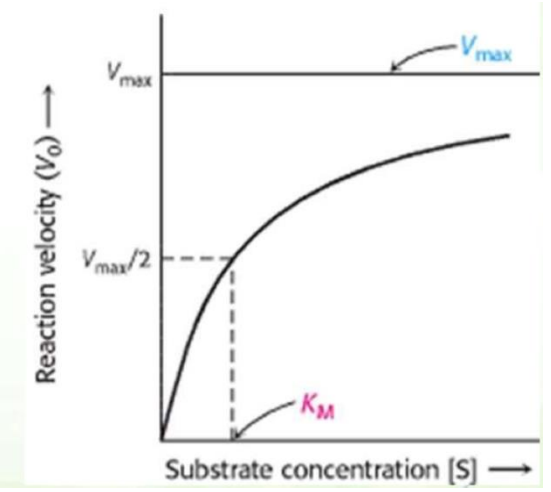
$$V_{max} = k_2[E]T = Kcat[E]_T$$

When we discuss Vmax, we are dealing with the total enzyme concentration $[E]_T$, not the free enzyme.
=>At Vmax, all enzyme molecules are bound to substrate, forming the enzyme-substrate complex (ES). There is effectively no free enzyme available.
=>Therefore, Vmax reflects the situation where all enzyme molecules are saturated and actively converting substrate to product.

The Michaelis constant (K_m)

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

- The lower the K_m of an enzyme towards its substrate, the higher the affinity
- When more than one substrate is involved? Each will have a unique K_m & V_{max}
- K_m values have a wide range. Mostly between (10^{-1} & 10^{-7} M)



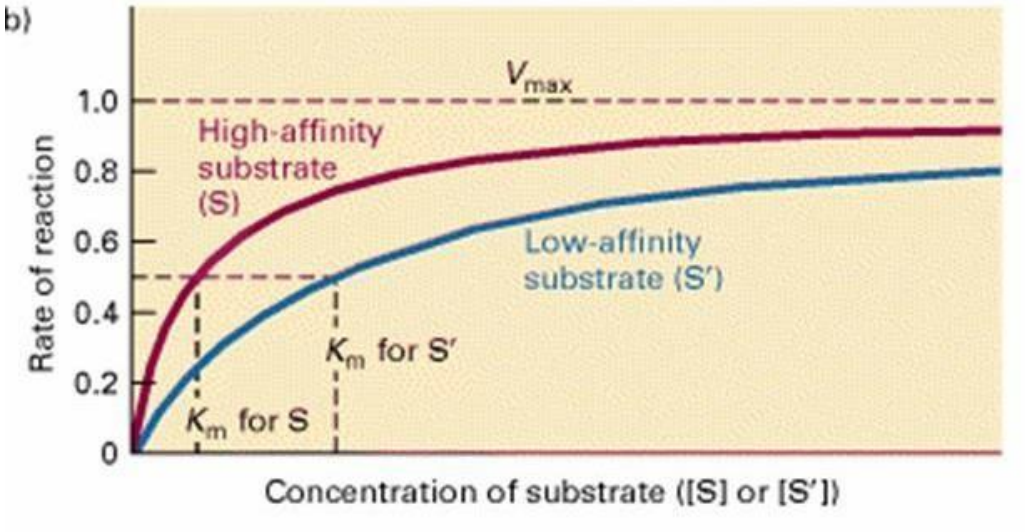


table 8–6

K_m for Some Enzymes and Substrates

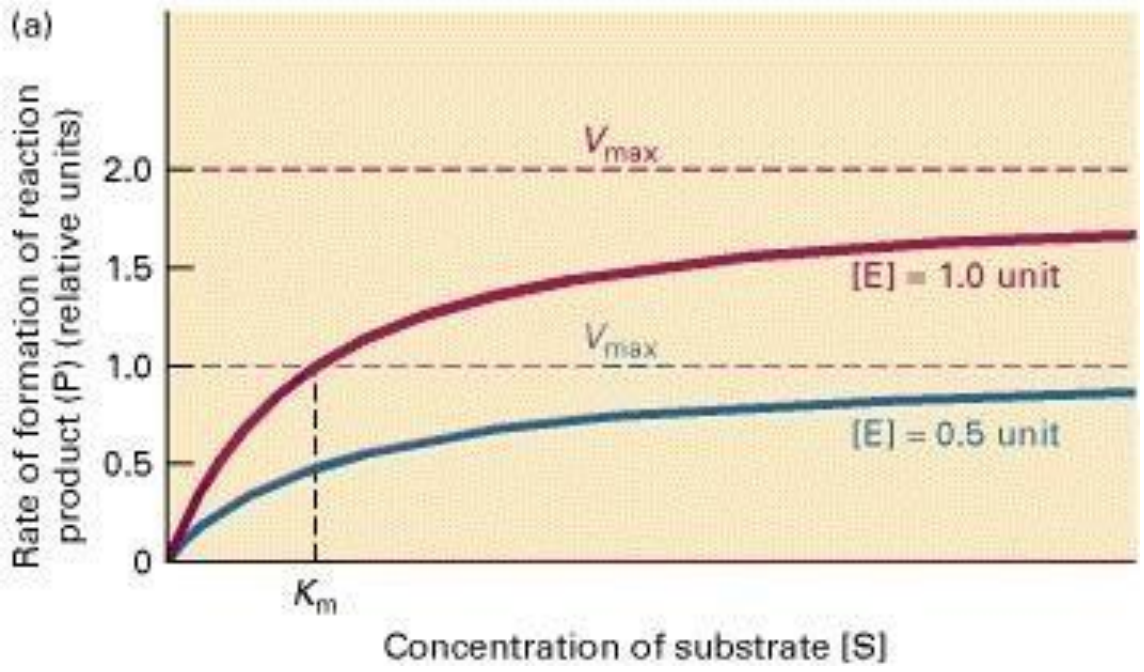
Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

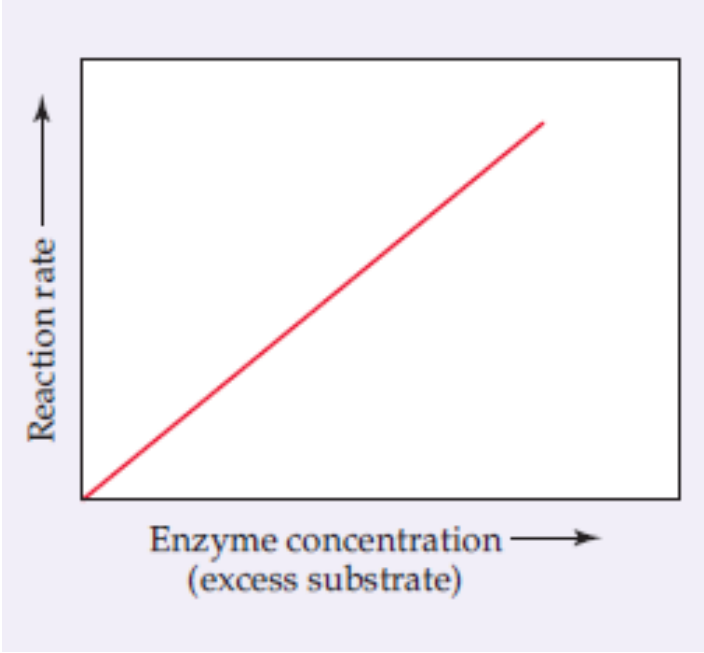
K_M & K_D
[E], K_M & V_{max}

- K_D: dissociation constant, The actual measure of the affinity
- $K_D = (k_{-1}/k_1)$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- When you increase the enzyme concentration, what will happen to V_{max} & K_m?





V_{max} & k_{cat}

Turnover Numbers (k_{cat}) of Some Enzymes		
Enzyme	**Substrate**	**k_{cat} (s^{-1})**
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

- For the enzymatic reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- The maximal rate, V_{max}, is equal to the product of k_2, also known as k_{cat}, and the total concentration of enzyme

$$V_{max} = k_2 [E]_T$$

- k_{cat}, the turnover number, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated

$$k_{cat} = V_{max} / [E]_T$$

- In other words, the maximal rate, V_{max}, reveals the turnover number of an enzyme if the total concentration of active sites $[E]_T$ is known

Example

- a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate
  - ✓ Hence, k_{cat} is 6×10^5 s^{-1}
  - ✓ 3.6×10^7 min^{-1}
- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is 1.7 µs for carbonic anhydrase
- The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^4 per second

Specificity & Efficiency

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{k_{cat}[E_T][S]}{K_M + [S]} \qquad V = (k_{cat}/K_M)[E][S]$$

- **Specificity constant (k_{cat}/K_M): determines the relative rate of the reaction at low [S]**
- **k_{cat}/K_M (M^{-1} min^{-1}) is indicative of:**
  - ✓ **Enzyme's substrate specificity: the higher the ratio, the higher the specificity**
  - ✓ **Enzyme's catalytic efficiency: the higher the ratio, the more efficient the enzyme**

Table 6.2

Turnover Numbers and Km for Some Typical Enzymes

Enzyme	Function	k_{cat} = Turnover Number*	K_M**
Catalase	Conversion of H_2O_2 to H_20 and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

k_{cat} values vary over a wide range K_M values also vary over a wide range K_{cat}/K_M, the range is only 4

When we look at the values of Kcat and Km for enzymes, we notice that they can vary widely. To compare enzymes more effectively, we can divide Kcat by Km.

This ratio is called the specificity constant:

K_{cat}/K_M

- **A high specificity constant indicates either:**
  1. A high Kcat (high catalytic activity), or
  2. A low Km (high binding affinity for the substrate)
- **A low specificity constant indicates either:**
  1. A low Kcat (slow enzyme), or
  2. A high Km (low binding affinity for the substrate)

The specificity constant typically ranges over 4 orders of magnitude, allowing us to compare enzymes more easily on a relative scale.

Enzyme kinetics parameters allow us to compare enzymes systematically. So far, we have discussed several key parameters(Vmax,Km,Kcat , Specificity constant(the ratio of Kcat to Km))

Other important parameters:

. **Reaction rate (υ) =>**the change in substrate concentration over time:

. **Enzyme activity =>** the change in the moles per unit time

=> The difference between change in concentration and enzyme activity is the volume. Multiplying by the reaction volume converts concentration change into actual enzymatic activity.

Enzyme activity = rate of reaction × reaction volume

. **Specific activity =>**used in enzyme purification to determine enzyme purity. It is defined as:

If you know the enzyme mass and enzyme activity, you can calculate specific activity to compare purity across preparations.

Turnover number is the same as k2 or Kcat.

· Starting from Vmax:

$$K_{cat} = \frac{V_{\max}}{[E]_T}$$

· If enzyme is expressed as mass, convert [E]T to moles using molecular weight (MW):

$$[E]_T = \frac{\text{mass of enzyme}}{\text{MW} \times \text{volume}}$$

· Substituting into the formula gives:

Remember: Enzyme activity = rate of reaction × reaction volume and Specific activity = enzyme activity / actual mass of enzyme

$$K_{cat} = \frac{V_{\max} \times \text{volume} \times MW}{\text{mass of enzyme}} \longrightarrow$$

Kcat= specific activity × molecular weight of enzyme

Reaction rate (v); Enzyme activity; Specific activity; Turnover number

- Reaction rate; measures the concentration of substrate consumed (or product produced) per unit time (mol/{L.s} or M/s)
- Enzyme activity; measures the number of moles of substrate consumed (or product produced) per unit time (mol/s)
  - ✓ Enzyme activity = rate of reaction × reaction volume
- Specific activity; measures moles of substrate converted per unit time per unit mass of enzyme (mol/{s.g})
  - ✓ Specific activity = enzyme activity / actual mass of enzyme
  - ✓ This is useful in determining enzyme purity after purification
- Turnover number; measures moles of substrate converted per unit time per moles of enzyme (min^{-1} or s^{-1})
  - ✓ Turnover number = specific activity × molecular weight of enzyme

Sample calculations

- A solution contains initially $25X10^{-4}$ mol L^{-1} of peptide substrate and 1.5 µg chymotrypsin in 2.5 ml volume. After 10 minutes, $18.6X10^{-4}$ mol L^{-1} of peptide substrate remain. Molar mass of chymotrypsin is 25,000 g mol^{-1}.

Pay attention when solving any question to the unit. You don't need calculators in the exam. You should know how to convert units.

- **How much is the rate of the reaction?**
  - **(conc./time)**
- **How much is the enzyme activity?**
  - **(mol./time)**
- **How much is the specific activity?**
  - **(enz. Act. / enz. Mass)**
- **How much is the turn over number?**
  - **(sp. Act. X enz. molar mass)**

Michaelis and Menten's equations represented a major breakthrough in enzymology. Before their work, studying enzymes and determining their kinetic parameters such as Vmax and Km was extremely difficult and required a lot of time, effort, money, and resources. To construct a plot and determine Vmax, one had to perform multiple experiments at different substrate concentrations. In each experiment, a specific substrate concentration was used, and the initial reaction velocity (V) was measured. This process had to be repeated many times to collect enough data points. These points were then plotted and aligned mathematically to estimate the maximum velocity, Vmax, which was inherently challenging due to the extensive work and precision required.

· To simplify, scientists Lineweaver and Burk took the reciprocal of the Michaelis-Menten equation: $v = \frac{V_{max} \cdot [S]}{[S] + K_m}$

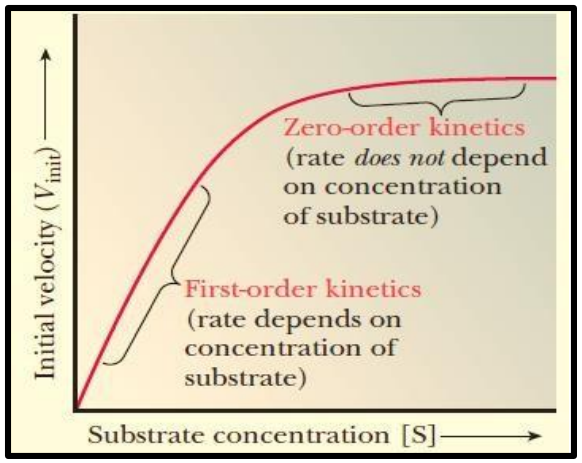
· Reciprocal gives a linear equation: $\frac{1}{v} = \left[\frac{K_m(1)}{V_{max}[S]} + \frac{1}{V_{max}}\right]$

· This is a straight line, which allowed:
· Slope = Km / Vmax
· Y-intercept = 1 / Vmax→ calculate Vmax
· X-intercept = -1 / Km → calculate Km

=> This transformation greatly reduced the effort, cost, and time needed to determine enzyme parameters.
=> It was a major breakthrough at the time, allowing researchers to estimate Vmax and Km without performing numerous exhaustive experiments.
=>Today, with computers and software, we can fit the Michaelis-Menten equation directly, so Lineweaver-Burk plots are less commonly used.

Disadvantage of Michaelis-Menten equation & Lineweaver-Burk or double-reciprocal plot

- Determining the K_m from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach V_{max}
- This prevents the calculation of both V_{max} & K_m
- Lineweaver-Burk plot: A plot of $1/v_o$ versus $1/[S]$ (double-reciprocal plot), yields a straight line with an y-intercept of $1/V_{max}$ and a slope of K_M/V_{max}
- The intercept on the x-axis is $-1/K_M$



$$v = \frac{V_{max} \cdot [S]}{[S] + K_m}$$

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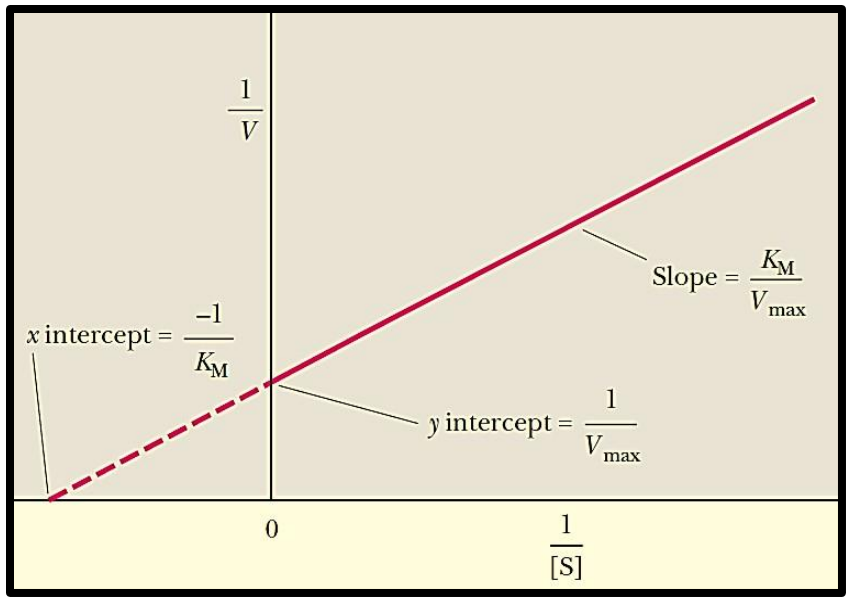
$$\frac{1}{v} = \left[\frac{K_m(1)}{V_{max}[S]} + \frac{1}{V_{max}}\right]$$



Example

- A biochemist obtains the following set of data for an enzyme that is known to follow **Michaelis-Menten kinetics**. **Approximately**, V_{max} of this enzyme is … & K_m is …?
  - A. 5000 & 699
  - B. 699 & 5000
  - C. 621 & 50
  - D. 94 & 1
  - E. 700 & 8

Substrate Concentration (μM)	Initial velocity (μmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

- You are working on the enzyme "Medicine" which has a molecular weight of 50,000 g/mol. You have used 10 μg of the enzyme in an experiment and the results show that the enzyme converts 9.6 μmol per min at 25°C. the turn-over number (k_{cat}) for the enzyme is:

A. 9.6 s^{-1} B. 48 s^{-1} C. 800 s^{-1}
D. 960 s^{-1} E. 1920 s^{-1}

Pay attention to the highlighted words:

Michaelis-Menten kinetics: the enzyme follows Michaelis-Menten kinetics means that its behavior is predictable according to the standard enzyme kinetic model

Approximately: No need for a calculator

Vmax is around 699, small deviations,such as 710 or 619are all acceptable within practical experience. The key point is to use the data to get a reasonable approximation of the kinetic parameters, rather than attempting a mathematically exact value.

Enzymes Regulation

Enzymes Regulation

Enzymes need to be **very highly regulated**, because their catalytic power is extremely high. To explain this, the doctor compared enzymes to cars: a regular car versus a racing car.

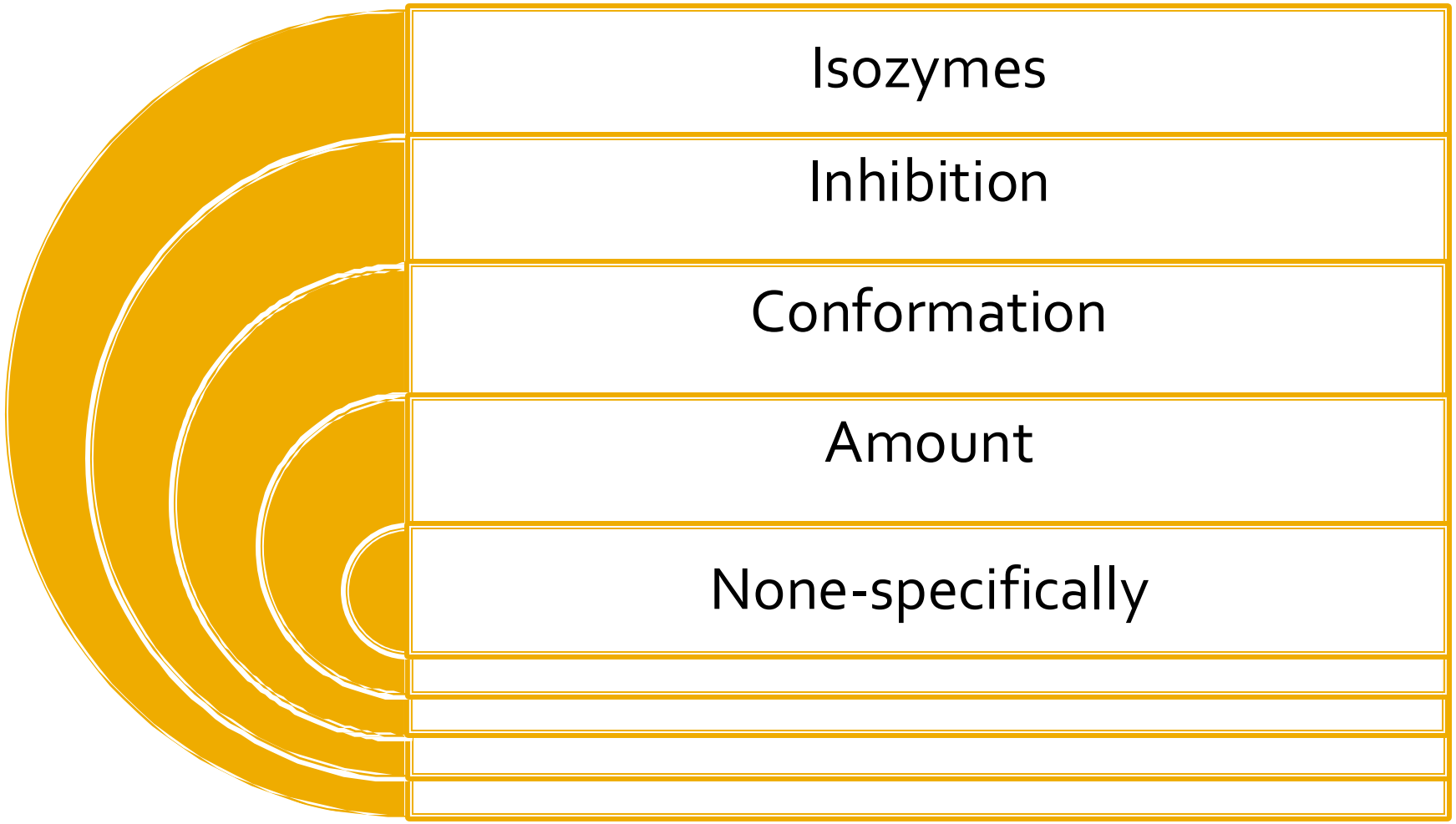
- A regular car doesn't require very strict regulation; if you make a small mistake, it might not be dangerous.
- But a racing car moves at very high speed, so a tiny mistake can cause a disaster.

Similarly, enzymes are like "racing cars." For example, if we say an enzyme has a Kcat = 40 million, this means it can catalyze 40 million reactions per second. That is an enormous speed, so the enzyme must be precisely regulated, otherwise it could cause chaos in the cell.

~> Enzymes are regulated by different modes of control.

- **Not all enzymes** are regulated by the same mechanism.
- **Some enzymes** are regulated by one way, others by another.
- **There is no enzyme** that is not regulated.
- **But there is also no enzyme** that is regulated by all possible mechanisms at once.

Modes of regulation



Isozymes

Isozymes are enzymes that have the same (Iso) function. They catalyze the same reaction, acting on the same substrate and producing the same product. However, they are generated from different genes, which means they have different amino acid sequences and therefore different structures.
=> We need isozymes because the same biochemical reaction may be required in different tissues under different conditions. By default, if there are isozymes, they must have different tissue localization.
>>Do they catalyze in the same way? No, because their amino acid sequences differ.

>>Do they bind to the same substrate to produce the same product? Yes, but with different kinetic parameters. A single amino acid change can alter substrate affinity (Km or Kd), catalytic efficiency (Kcat), or overall enzymatic activity.

=> Isozymes may also differ in their regulation. For example, one isozyme can be inhibited or activated by a certain molecule because it has the proper amino acid residues at the binding site, while another isozyme lacks those residues and is therefore unaffected. This means that one isozyme can be inhibited while another continues to function, allowing the cell to fine-tune metabolism according to tissue needs.

Hexokinase is not just one enzyme ;in fact, we have many copies in the body, around 24-25 isoforms. But usually, we compare two important ones. One is **located in the liver and pancreas, called Hexokinase IV (also known as glucokinase). The other is located in red blood cells (RBCs), called Hexokinase I.**

The difference between these isozymes arises from amino acid substitutions, which cause differences in their kinetic parameters. Both enzymes catalyze the same reaction: the phosphorylation of glucose to glucose-6-phosphate (G6P).

Why is this step important? Because phosphorylation adds a phosphate group (a large, polar, and negatively charged molecule). This prevents glucose from leaving the cell again through the glucose transporters (GLUTs). In other words, phosphorylation traps glucose inside the cell. No matter what pathway glucose will enter { glycogen synthesis, glycolysis to pyruvate, or the pentose phosphate pathway to ribose } the first essential step is phosphorylation.

The kinetic parameters:

- Hexokinase I (RBCs): Km ≈ 0.1 mM → very high affinity for glucose.
- Hexokinase IV (liver, pancreas): Km ≈ 10 mM → much lower affinity.

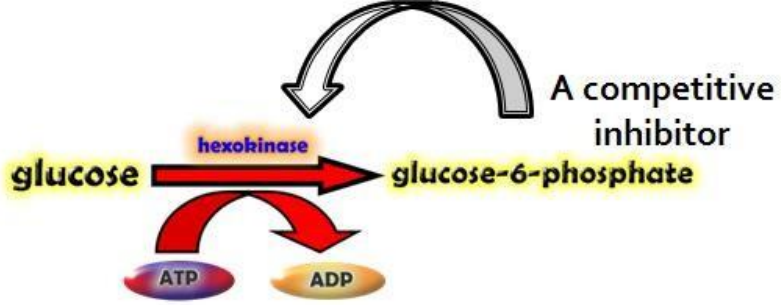
This means that Hexokinase I will function efficiently even at very low glucose concentrations (below normal fasting levels, ~5 mM). This is crucial because RBCs have no mitochondria, so they rely entirely on glycolysis for ATP. Even close to starvation, RBCs must still be able to phosphorylate glucose.

In contrast, Hexokinase IV (glucokinase) in the liver and pancreas requires higher glucose concentrations to work effectively. During fasting, when blood glucose is low, glucokinase activity is minimal. But after a meal, when glucose rises above its Km, glucokinase becomes active, promoting glucose storage (glycogen synthesis) or sensing glucose levels in the pancreas.

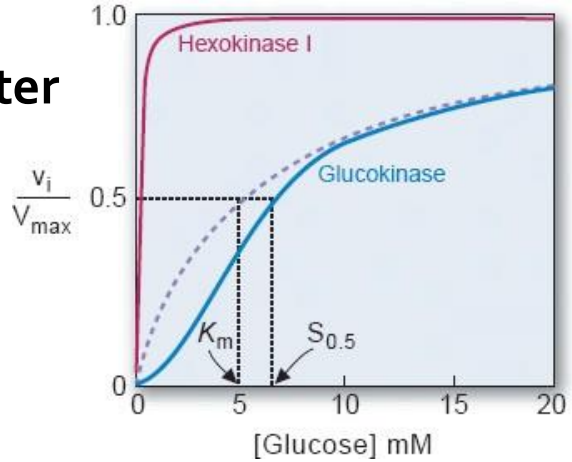
When glucose enters the cell, hexokinase adds a phosphate group on carbon-6 to form glucose-6-phosphate (G6P). This product is close in structure to glucose, so it can fit into the active site and act as a competitive inhibitor. In red blood cells, hexokinase I is inhibited by G6P, and this regulation is important because RBCs rely only on glycolysis for energy. If enough product accumulates, the enzyme must stop. In contrast, in the liver and pancreas, hexokinase IV (glucokinase) is not inhibited by G6P. The reason is that in the liver excess glucose should be stored, so the pathway continues into glycogenesis. In the pancreas, glucokinase acts as a mirror of blood glucose concentration, so it must keep working even when glucose is very high. This difference is due to changes in amino acids, which affect Km and regulation.

Note:issues of regulation,whether inhibition or activation become important when glucose concentration is very high, above the needs of the tissue.

1. Isozymes (isoenzymes) The Differential K_M Value "Hexokinase"



- What are isozymes? Same substrate & product, different gene, different localization, different parameters (K_m, V_{max}, k_{cat})
- Hexokinase found in RBCs & in liver
- Catalyzes the first step in glucose metabolism
- Hexokinase I (RBCs): K_M (glucose) ≈ 0.1 mM
- Hexokinase I V (glucokinase, liver, pancreas) ≈ 10 mM
- RBCs: when blood glucose falls below its normal fasting level (≈ 5 mM), RBCs could still phosphorylate glucose at rates near V_{max}
- Liver: rate of phosphorylation increases above fasting levels (after a high- carbohydrate meal)
  - High K_M of hepatic glucokinase promotes storage of glucose
- Pancreas: works as a sensor



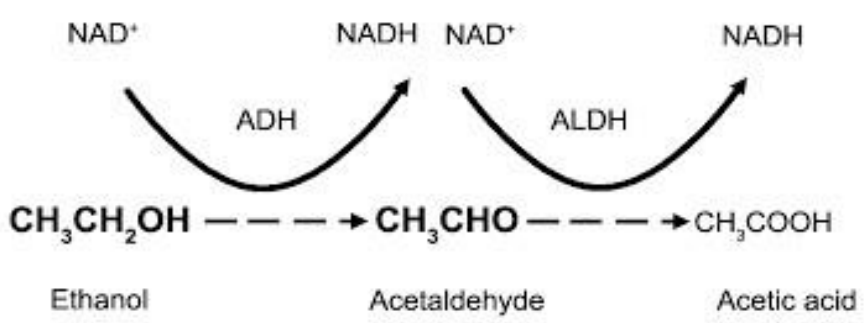
Lactate Dehydrogenase (LDH) ~> **<u>according to the doctor it is not included :</u>**

LDH is a tetramer enzyme with 5 isoforms. Some isoforms exist in the brain, some in heart muscle, skeletal muscle, and RBCs. The subunits can be H4, M4, or combinations in between, depending on the tissue.

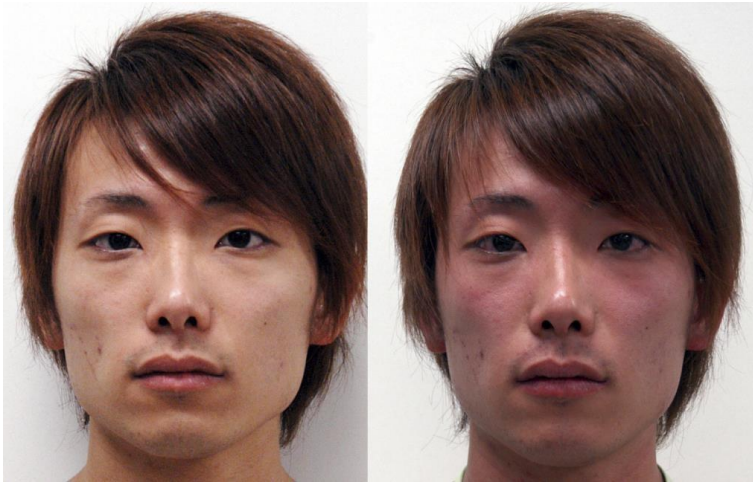
Aldehyde Dehydrogenase (ALDH):

There are 2 main copies: one mitochondrial (ALDH2) and one cytosolic (ALDH1). This is how alcohol is processed in the body. Differences in enzyme activity explain why some people feel stronger effects of alcohol: some copies may be missing or less active. The mitochondrial ALDH2 has low Km, so it binds acetaldehyde (the toxic form of alcohol) with high affinity and breaks it down efficiently. If acetaldehyde accumulates above mitochondrial capacity, it is then processed by the cytosolic ALDH1, which has lower affinity. Accumulation of acetaldehyde causes toxicity, producing flushing, tachycardia, and dizziness.

Aldehyde dehydrogenase (ALDH)



- Oxidation of acetaldehyde to acetate.
- Four tetrameric isozymes (I-IV)
- ALDH I (low Km; mitochondrial) and ALDH II (higher Km; cytosolic)
- ~50% of Japanese & Chinese are unable to produce ALDH I (acetaldehyde accumulates in the cytosol. If the concentration becomes too high, it can spill out of the cells and tissues into the systemic circulation, which makes the effects of alcohol much stronger in these individuals compared to others)
- (not observed in Caucasian & Negroid populations)
  - Flushing response
  - Tachycardia



2. Inhibition

NEXT MODIFIED ..

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

أقرب ما تكون من الله وأنت ساجد
وأقرب السجود في وقت السحر
فمن أثقله الذنب، أو أحاط به الكرب
فليسجد في السحر، ويُلحّ بدعاء الواثق
فالقرب في ذروته، والإجابة على الوعد

بكامل هشاشتك وتعبك، قال لك "اقترب"
بكامل ضعفك ومخاوفك، علمك "اقترب"
بكل ما فيك من هواجس وأفكار، هداك "اقترب"
بكل مساوئ روحك وعيوبك، عرفّك "اقترب", وإن تاهت بكَ الطرق، لقد دلّك عليه "اقترب"
في قلقك وضعيف انتباهك، ناداك ربك "اقترب"
قال "اقترب" أتظن بعد هذا القرب اغتراب؟

ولعظمة ما في السجود قال نبينا ﷺ لمن سأله الجنة أن يعينه بكثرة السجود.
عن ربيعة بن كعب الأسلمي رضي الله عنه قال: "كنت أبيت مع رسول ﷺ فأتيته بوَضوئه وحاجته، فقال لي: سل، فقلت: أسألك مرافقتك في الجنة، فقال: أَوْ غيرَ ذلك؟، قلت: هو ذاك، قال: فأعني على نفسك بكثرة السجود" . [رواه مسلم]