COURSE MSc (BIOTECHNOLOGY) III SEM

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BISUBSTRATE REACTIONS

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BISUBSTRATE REACTIONS

- Enzymatic reactions involving two substrates and yielding two Products are called **bisubstrate reactions.**
- **Bisubstrate reactions** account for 60% of known biochemical reactions.

$$A + B \overset{E}{\rightleftharpoons} P + Q$$

- These are either **transferase** reactions in which the enzyme catalyzes the transfer of a specific functional group, X, from one of the substrates to the other: or **oxidation–reduction reactions** in which reducing equivalents are transferred between the two substrates.

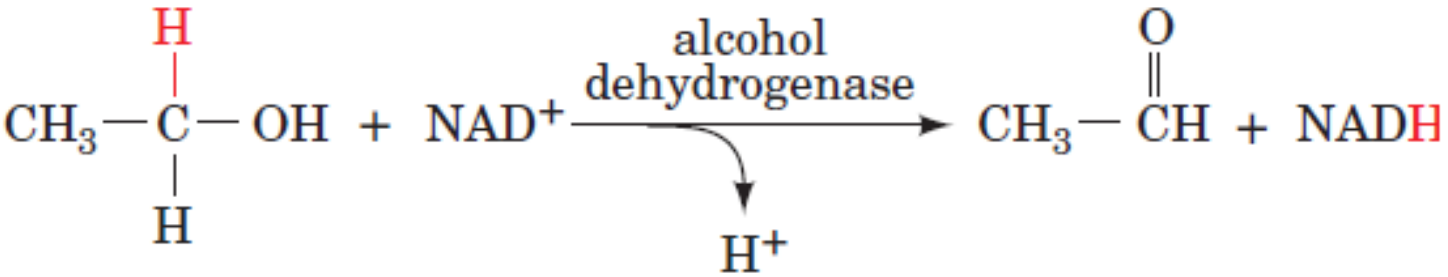
$$P{-}X + B \overset{E}{\rightleftharpoons} P + B{-}X$$

BISUBSTRATE REACTIONS

(a)

Polypeptide

(b)



BISUBSTRATE REACTIONS

Terminology

The nomenclature system introduced by W.W. Cleland for representing enzymatic reactions:

1. Substrates are designated by the letters A, B, C, and D *in the order that they add to the enzyme.*

2. Products are designated P, Q, R, and S *in the order that they leave the enzyme.*

3. Stable enzyme forms are designated E, F, and G with E being the free enzyme, if such distinctions can be made. A stable enzyme form is defined as one that by itself is incapable of converting to another stable enzyme form

4. The numbers of reactants and products in a given reaction are specified, in order, by the terms **Uni** (one), **Bi** (two), **Ter** (three), and **Quad** (four). A reaction requiring one substrate and yielding three products is designated a Uni Ter reaction. In this section, we shall be concerned with reactions that require two substrates and yield two products, that is, Bi Bi reactions. Keep in mind, however, that there are numerous examples of even more complex reactions.

BISUBSTRATE REACTIONS

Types of Bi Bi Reactions

Enzyme-catalyzed group-transfer reactions fall under two major mechanistic classifications:

1. Sequential Reactions: *Reactions in which all substrates must combine with the enzyme before a reaction can occur and products can be released are known as* ***Sequential reactions.*** In such reactions, the group being transferred, X, is directly passed from A (P—X) to B, yielding P and Q (B—X). Hence, such reactions are also called **single-displacement reactions.**

Sequential reactions can be subclassified into those with a compulsory order of substrate addition to the enzyme, which are said to have an **Ordered mechanism,** and those with no preference for the order of substrate addition, which are described as having a **Random mechanism.**

In the Ordered mechanism, the binding of the first substrate is apparently, required for the enzyme to form the binding site for the second substrate, whereas for the Random mechanism, both binding sites are present on the free enzyme.

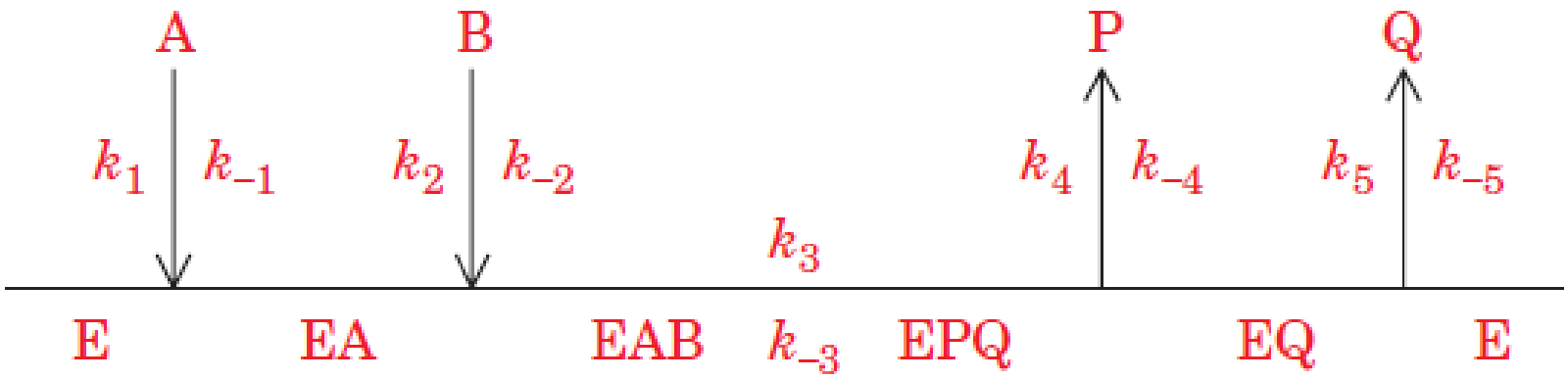
BISUBSTRATE REACTIONS

Cleland's shorthand notation

The enzyme is represented by a horizontal line and successive additions of substrates and release of products are denoted by vertical arrows.

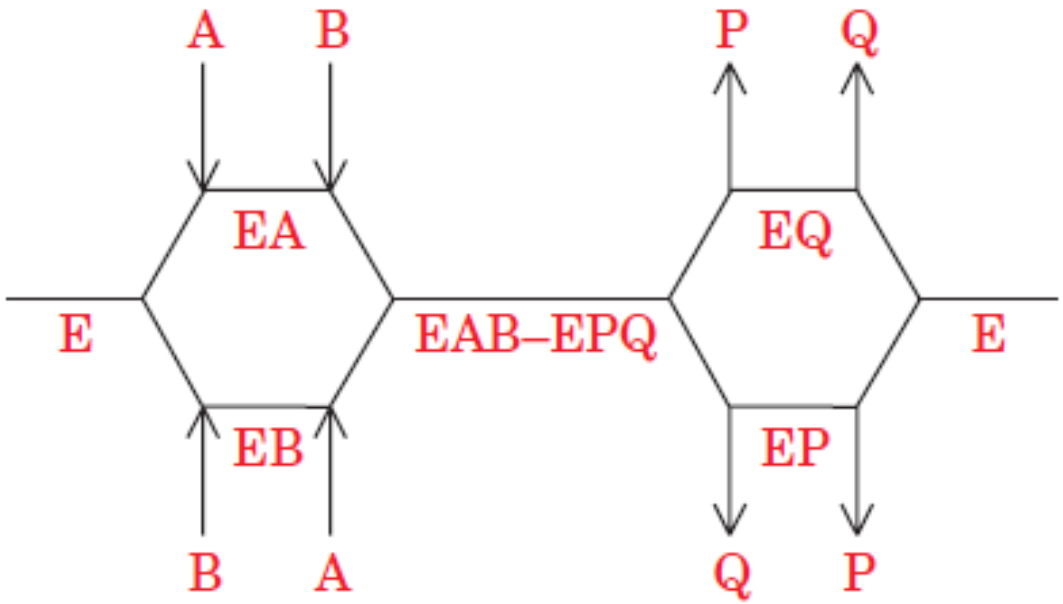
Enzyme forms are placed under the line and rate constants, if given, are to the left of the arrow or on top of the line for forward reactions.

An Ordered Bi Bi reaction



BISUBSTRATE REACTIONS

A **Random Bi Bi** reaction is diagrammed: Some dehydrogenases and kinases operate through Random Bi Bi mechanisms

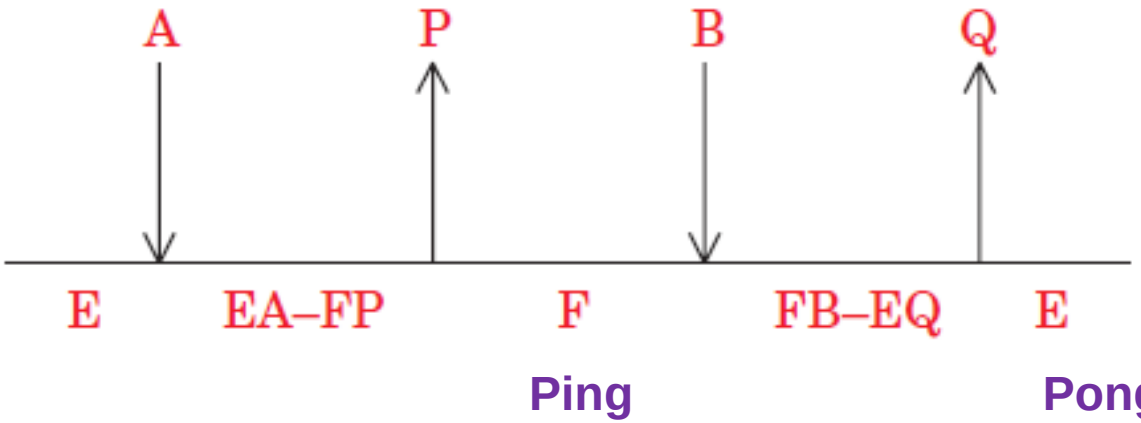


BISUBSTRATE REACTIONS

2. Ping Pong Reactions:

Mechanisms in which one or more products are released before all substrates have been added are known as ***Ping Pong reactions.*** The **Ping Pong Bi Bi** reaction is represented by:

Enzymes, like chymotrypsin, transaminases and some flavoenzymes, react with Ping Pong mechanisms.



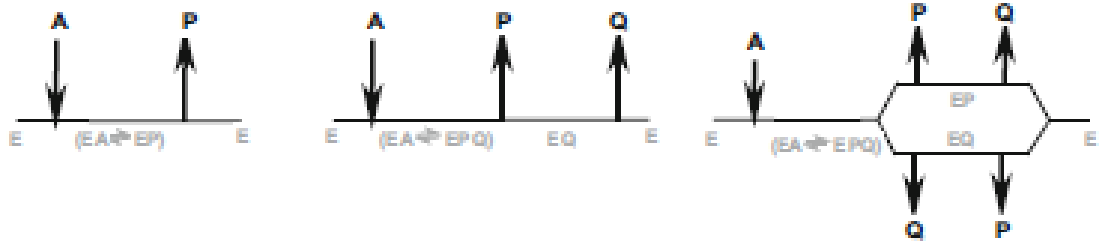
A functional group X of the first substrate A (P-X) is displaced from the substrate by the enzyme E to yield the first product P and a stable enzyme form F (E- X) is tightly bound to the enzyme (Ping).

In the second stage , X is displaced from the enzyme by B to yield the second product Q (B-X), thereby regenerating the original form of the enzyme, E (Pong).

Such reactions are therefore also known as **double-displacement reactions**

BISUBSTRATE REACTIONS KINETICS

Enzyme reactions with one substrate



Alanine racemase (EC 5.1.1.1)
A= L-Alanine
P= D-Alanine

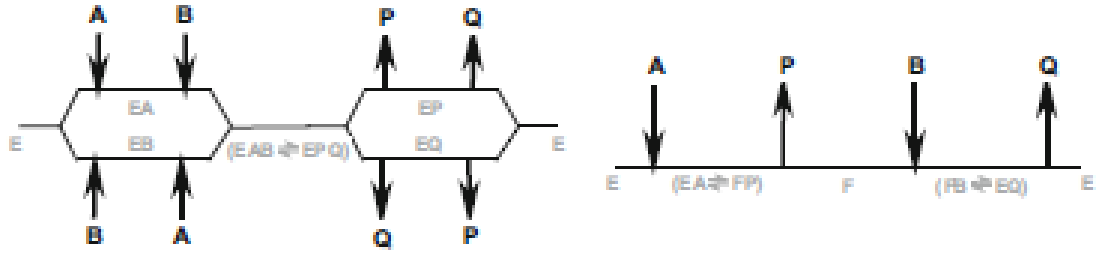
Isocitrate lyase (EC 4.1.3.1)
A= Isocitrate
P= Succinate, Q= Glyoxylate

Cytidine deaminase (EC 3.5.4.5)
A= Cytidine ($+H_2O$)
P/Q= Uridine/NH_3

Enzyme reactions with two substrates and two products

Malate dehydrogenase (EC 1.1.1.37)
A= NAD^+, B= Malate
P= Oxaloacetate, Q= NADH

ADP-glucose pyrophosphorylase (EC 2.7.7.27)
A= ATP-Mg, B= Glucose 1-phosphate
P= Pyrophosphate, Q= ADP-glucose

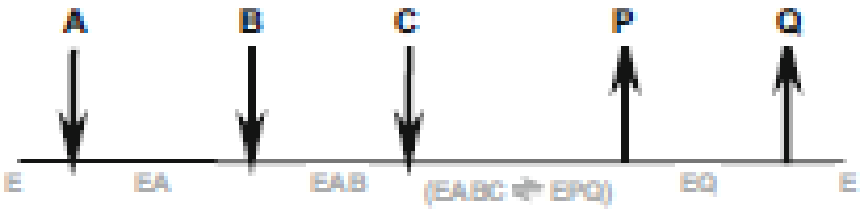


Alcohol dehydrogenase (EC 1.1.1.1)
A/B= NAD^+/2-Propanol
P/Q= NADH/Acetone

Nucleoside-diphosphate kinase (EC 2.7.4.6)
A= ATP-Mg, B= NDP-Mg
P= ADP-Mg, Q= NTP-Mg

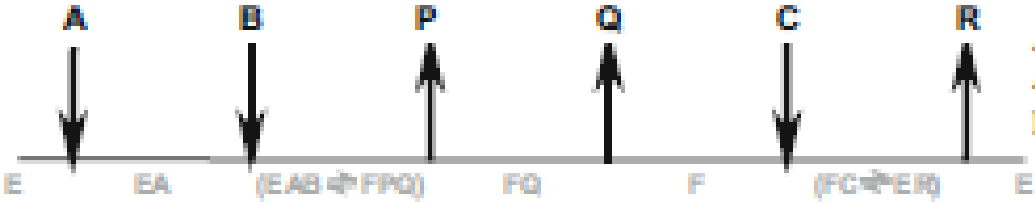
BISUBSTRATE REACTIONS KINETICS

Enzyme reactions with three substrates and two products



Glutamate dehydrogenase (EC 1.4.1.4)
A= NADPH, B= 2-Oxoglutarate, C= NH_3
P= Glutamate, Q= $NADP^+$

Glutamine synthetase (EC 6.3.1.2)
A= ATP-Mg, B= Glutamate, C= NH_3
P= Pi, Q= Glutamine, R= ADP-Mg



Acetyl CoA carboxylase (EC 6.4.1.2)
A= ATP-Mg, B= HCO_3^-, C= Acc CoA
P= PPi, Q= ADP-Mg, R= Mal CoA