Chapitre 3 : Two-substrate enzyme kinetics

Exercice 1

An enzyme catalyzes a reaction between two substrates:

$$A + B \longrightarrow C + D$$

In order to determine the kinetic mechanism of the reaction, the initial reaction rates were measured at varying substrate concentrations. The following results were obtained:

Concentration A*	***Concentration B****				
	0,1	0,2	0,5	1	2
0,1	0,05	0,1	0,21	0,33	0,46
0,2	0,09	0,16	0,3	0,43	0,55
0,5	0,14	0,24	0,4	0,52	0,62
1	0,17	0,28	0,45	0,57	0,65
2	0195	0,31	0,48	0,59	0,66

* Expressed in absorbance at 357 nm.

The reaction rates are expressed as the change in absorbance at 357 nm per minute. The molar extinction coefficients at 357 nm for the different compounds are as follows: $\varepsilon_A = 1350$, $\varepsilon_B = 225$, $\varepsilon_C = 720$ and $\varepsilon_D = 1830$

Determine the **mechanism of the reaction** and the **different kinetic constants** of the scheme (Vmax in moles·s^{-1} and the affinity constants).

Solution : Mécanisme ordonné ; A se fixe le premier.

2) $K_A = 3{,}1.10^{-4}$ M, constante d'affinité = $1/K_A = 3{,}2.10^3$ M^{-1}

$K_B = 1{,}1.10^{-3}$ M, constante d'affinité = $1/K_B = 0{,}9 .10^3$ M^{-1}.

$Vm = 0{,}77\ \Delta A/mn = 1{,}3.10^{-5}$ $M.s^{-1}$.

Exercice 2

The kinetic study of **malonyl-transacylase** was carried out to determine its mechanism of action. This enzyme catalyzes the transfer of the malonyl group from malonyl-coenzyme A to a sulfhydryl group of **ACP** (Acyl Carrier Protein).

The reaction can be monitored using malonyl-coenzyme A labeled with carbon-14. After two minutes of reaction, **perchloric acid** is added; ACP precipitates, and the radioactivity incorporated into the precipitate is measured.

The following table shows, for different substrate concentrations, the reaction rates expressed in **nanomoles of malonyl transferred per minute** when the experiment is performed in 0.2 mL, with an enzyme concentration of 5.98×10^{-10} mole/ liter.

(malonyl-CoA) (μM)	*ACP (acyl carrier protein) (μM)*		
	12,5	25	75
12,5	0,20	0,28	0,38
25	0,25	0,40	0,63
40	0,28	0,465	0,83
50	0,29	0,50	0,93
60	0,30	0,52	1,01

1/ Determine the **mechanism of the reaction** and the corresponding **kinetic parameters**.
2/ Calculate the **catalytic constant (k_{cat})** of the enzyme.

Solution : Mécanisme « ping-pong »
Vm = 11,3 nmoles/mn
Km (malonyl-CoA) = 290 μM
Km(ACP) = 400 μM
k_{cat} = 1600 s^{-1}.

Exercice 3

Chelatase is an enzyme that catalyzes the insertion of iron into porphyrins to form hemes; iron can be replaced by cobalt or zinc.

In the following experiment, the rate of cobalt incorporation into **protoporphyrin IX** was measured by spectrophotometry at different concentrations of cobalt and protoporphyrin. The following table shows these rates, expressed in **nanomoles of Co-heme formed per minute**:

(cobalt) *(μM)*	*(protoporphyrine) (μM)*			
	2,5	**3,3**	**5**	**10**
4	2,7	3,2	3,9	4,9
6	3,5	4,1	5	6,3
8	4,1	4,8	5,8	7,35
10	4,55	5,3	6,5	8,2

Determine the **mechanism of the reaction** and the corresponding **kinetic parameters**.

Solution : Mécanisme au hasard, fixation indépendante
Km(cobalt) = 8 μM
Km(protoporphyrine) = 3,6 μM
Vm = 20 nmoles/mn.