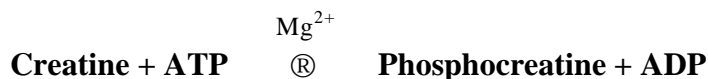


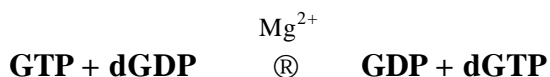
Microbial and Enzyme Kinetics Tutorial 5

The data below were obtained in a study of the following reaction catalysed by rabbit muscle creatine kinase



[ATP]/mM	0.46	0.62	1.23	3.68
[Creatine]/ mM	Velocity/ mol s ⁻¹			
6	0.377	0.463	0.660	0.968
10	0.555	0.678	0.950	1.308
20	0.845	1.005	1.338	1.803
40	1.180	1.378	1.718	2.295

- Deduce the kinetic mechanism of the reaction
 - Determine the kinetic parameters of the reaction
2. The reaction below is catalysed by the enzyme diphosphokinase



The following data was obtained from an experimental run

[GTP]/ μM	22	30	50	100
[dGDP]/ μM	Velocity/mol s ⁻¹			
20	0.095	0.112	0.141	0.196
25	0.102	0.120	0.155	0.223
40	0.112	0.136	0.180	0.284
100	0.125	0.156	0.218	0.385

- Plot graph of $1/v$ vs $1/[\text{dGDP}]$ for each value of $[\text{GTP}]$ on the same axes. Determine the slope and intercept of each line.
- Deduce the kinetic mechanism of the reaction
- Determine the kinetic parameters of the reaction

To determine kinetics for two substrate systems

For a reaction



1. Plot values of $1/V$ vs $1/[A]$ for various values of $[B]$. This will give a series of lines. Measure the slope and intercept of each line.
 - If the slope of the lines varies significantly, the mechanism involves a ternary complex
 - If the lines are parallel, the mechanism is “ping pong”
2. For a ternary complex mechanism
 - a. Plot a graph of slope vs $1/[B]$ from the data in (1) above. This should give a straight line, slope $K'_A K_B / V_{max}$ and intercept K_A / V_{max} .
 - b. Plot a graph of Intercept vs $1/[B]$ from the data in (1) above. This should give a straight line of slope K_B / V_{max} and intercept $1 / V_{max}$.
3. For the ping-pong mechanism, each line will have a slope K_A / V_{max} .
 - a. Plot a graph of intercept vs $1/[B]$ to give a line of slope K_B / V_{max} and intercept $1 / V_{max}$.
 - b. Use this plot to calculate V_{max} , hence determine K_A and K_B from the slopes of the lines in the two graphs