UNIVERSITY OF TEESSIDE

School of Science and Technology

Microbial and Enzyme Kinetics Tutorial 5

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

$$\textbf{Creatine + ATP} \xrightarrow{Mg^{2+}} \textbf{Phosphocreatine + ADP}$$

[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

a. Deduce the kinetic mechanism of the reaction

b. Determine the kinetic parameters of the reaction

2. The reaction below is catalysed by the enzyme diphosphokinase

$$\textbf{GTP + dGDP} \xrightarrow{Mg^{2+}} \textbf{GDP + dGTP}$$

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

a. Plot graph of 1/v vs 1/[dGDP] for each value of [GTP] on the same axes. Determine the slope and intercept of each line.

b. Deduce the kinetic mechanism of the reaction

c. Determine the kinetic parameters of the reaction

To determine kinetics for two substrate systems

For a reaction

$$A + B \Leftrightarrow P + Q$$

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