BIOC1015: Kinetics of alkaline phosphatase inhibition

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Contents

1 Introduction

Alkaline phosphatase catalyses the hydrolysis of p-nitrophenylphosphate to p-nitrophenol, which absorbs at 405nm. This reaction was used to investigate the inhibition of alkaline phosphatase by inorganic phosphate.

The experiment was conducted in two parts:

1. A trial run: testing the reaction and its rate in the presence and absence of inhibitor, and designing an assay protocol to allow the reaction to be accurately followed and the rate determined.
2. The assays themselves.

A Lineweaver-Burk plot was used to interpret the results and verify Michaelis-Menten kinetics.

2 Part 1: Trial run

Two reactions were carried out as detailed in the manual.

The concentrations of substrate (p-nitrophenyl phosphate), enzyme (calf intestinal alkaline phosphatase (CIAP)) and inhibitor (sodium hydrogen phosphate) were 0.02M, 35mg/l and 0.04M respectively.

The reaction mixtures were as follows:

1. 1.0ml buffer, 2.0ml water, 0.5ml substrate, 0.5ml enzyme.
2. 1.0ml buffer, 1.5ml water, 0.5ml substrate, 0.5ml enzyme, 0.5ml inhibitor.

Readings were taken for 5 minutes and a graph plotted (graph 1). The initial rates of reaction were found to be 0.119 Abs/min and 0.064 Abs/min respectively.

The rates of reaction and amount of inhibition (approximately 50%) were considered satisfactory for the main experiment.

Ten assays were planned (five with inhibitor, five without), with the amount of substrate varying between 0.2 and 1.0ml. The amount of enzyme and inhibitor (where present) were kept at 0.5ml, and the total reaction volume kept at 4.0ml. The substrate concentrations were chosen to ensure equal spacing along the abscissa on the final double-reciprocal (Lineweaver-Burk) plot.

3 Part 2: Assays

3.1 Procedure

10 test tubes were prepared as shown in table 1, with the exception of enzyme. The tubes were heated to 30°C, enzyme was added and the absorbance at 405nm measured after 30 seconds and at 30-second intervals thereafter.

For tubes 5 and 10, due to the speed of reaction, extra measurements were taken at 15-second intervals. This was probably unnecessary.

Due to an oversight, the reactions in tubes 1-3 were carried out at room temperature instead of at 30°C. There was insufficient time for repeats, so it is hoped that this did not interfere unduly with the reaction rate.

3.2 Results

The data (table 2) were plotted (graphs 2-6), and the initial reaction rates (v_0) calculated from the gradients (table 3). A table of reciprocals (table 4) was calculated and a Lineweaver-Burk plot of $1/v_0$ against $1/[S]$ was produced (graph 7).

The reactions with inhibitor present progressed more slowly, indicating inhibition was taking place. The data fit reasonably well to straight lines, indicating Michaelis-Menten kinetics.

	$1/v_{max}$ (min/Abs)	v_{max}(Abs/min)	$1/K_m$ (M^{-1})	K_m (mM)
Without inhibitor	4.0	0.25	300	3.3
With inhibitor	5.2	0.19	150	6.7

Table 5: interpretation of the Lineweaver-Burk plot

The intersection of the lines of best fit did not occur on either of the axes, thus v_{max} and K_m were both different (table 5). This is not necessarily indicative of inhibition that is neither competitive nor non-competitive, as the errors in the data were substantial (the points were not close to the lines of best fit). The two v_{max} values were much closer than than the two $K_m$values: if the difference was purely due to experimental error this would indicate competitive inhibition.

From a biochemical perspective this would be unsurprising, as an obvious possible reaction mechanism would involve an active site that binds phosphate groups non-selectively prior to removing them (and that would thus be inactivatable by inorganic phosphate, which would compete for binding at the active site).

4 Conclusion

The effects of inorganic phosphate as an inhibitor of alkaline phosphatase were measured, and kinetic constants calculated from a Lineweaver-Burk plot.

For an enzyme concentration of 4.4mg/l, K_m values of 3.3mM (no inhibitor) and 6.7mM (with 5mM phosphate inhibitor) were observed.

The respective v_{max} values were 0.25 and 0.19 Abs/min; while $Abs_{405} \propto [product]$, the constant of proportionality is unknown as the spectophotometer was not calibrated, thus the activity of the enzyme (in terms of amount of substrate converted to product per unit time) cannot be calculated.

While these results are not clear-cut, they are suggestive of competitive inhibition, which would support the idea of the enzyme's active site binding phosphate groups.