Lab biochemistry

Science, Chemistry

Determination of the Kinetic constants, Km, Kcat and Vmax of yeast alcohol dehydrogenase (ADH) with three alcohol substrates (ethanol, propan ol and butan-1-ol).

Introduction

The objective of this experiment is to find the Km, kcat and Vmax values of yeast alcohol dehydrogenase. We used ethanol, propan-1-ol and butan-1-ol to carry out this experiment.

With a molecular weight of 150. 000, yeast dehydrogenase is classified as an NAD linked enzyme. There are four subunits within NAD which consist of Zn2+ ions, the purpose of which is to aid in transfereing a hydride ion from the alcohol to NAD+ .

The overall reaction equation is :

RCH2OH + NAD+ ☂ RCHO + NADH + H+

Method

As written in the lab protocol sheet

Results

Table 1

Ethanol

Time

Absorbance values for Substrate concentrations

Seconds

Minutes

0. 080

0. 040

0. 020

0. 010

0. 005

0. 000

0. 000

0. 000

0. 000

0. 000

0. 000

0. 000

0. 000

10. 000

0. 167

0. 267

0. 120

0. 081

0. 073

0. 035

20. 000

0. 333

0. 369

0. 165

0. 126

0. 133

0. 061

30. 000

0. 500

0. 439

0. 264

0. 169

0. 178

0. 098

40. 000

0. 667

0. 550

0. 318

0. 217

0. 216

0. 127

50. 000

0. 833

0. 600

0. 384

0. 258

0. 252

0. 155

60. 000

1. 000

0. 725

0. 439

0. 308

0. 287

0. 178

Fig. 1

Fig 1. 1

Fig 1. 2

Fig 1. 3

Fig 1. 4

Table 2

Propan-1-ol

Time

Absorbance values for Substrate concentrations

Seconds

Minutes

0. 08

0. 04

0. 02

0. 01

0. 005

0

0. 000

0. 000

0. 000

0. 000

0. 000

0. 000

10

0. 167

0. 018

0. 041

0. 036

0. 033

0. 038

20

0. 333

0. 053

0. 061

0. 044

0. 039

0. 040

30

0. 500

0. 085

0. 085

0. 062

0. 052

0. 044

40

0. 667

0. 115

0. 111

0. 079

0. 035

0. 052

50

0. 833

0. 143

0. 137

0. 097

0. 076

0. 058

60

1. 000

0. 168

0. 159

0. 112

0. 087

0. 063

70

1. 167

0. 196

0. 184

0. 128

0. 099

0. 071

Fig 1. 5

Fig 1. 6

Fig 1. 7

Fig 1. 8

Fig 1. 9

Table of absorbance values at 340nm using different substrate concentration for Butan-1-ol and the graphs to show this data.

Table 3

Butan-1-ol

Time

Absorbance values for Substrate concentrations

Seconds

Minutes

0. 08

0. 04

0. 02

0. 01

0. 005

0

0. 000

0. 000

0. 000

0. 000

0. 000

0. 000

10

0. 167

0. 043

0. 03

0. 029

0. 027

0. 024

20

0. 333

0. 078

0. 05

0. 048

0. 038

0. 032

30

0. 500

0. 119

0. 082

0. 074

0. 048

0. 036

40

0. 667

0. 161

0. 108

0. 083

0. 057

0. 041

50

0. 833

0. 193

0. 134

0. 098

0. 066

0. 047

60

1. 000

0. 23

0. 16

0. 116

0. 076

0. 051

70

1. 167

0. 264

0. 183

0. 13

0. 083

0. 054

Fig 2. 0

Fig 2. 1

Fig 2. 2

Fig 2. 3

Fig 2. 4

The graphs depicted above have been plotted for for 0. 080, 0. 040, 0. 020, 0. 010 and 0. 005M substrate concentrations. All of these graphs have absorbance (340nm) on the y-axis and Time (min) on the x-axis. The graphs were drawn individually however due to a great deal of overlap in the data, it was better to combine it into a single graph for each alcohol as it made the interpretation of the date considerably easier.

Michaelis Menten curves

Velocity calculation:

1) The linear line equations of the absorbance vs. Time plot. provided the gradient.

2) The gradient was divided by the Enzyme co-efficient which in this case was

6220 l/mol/cm

3) The above answer was multiplied by the volume of the cuvette which was 0. 003 (3ml÷1000)

4) The above answer was multiplied with 106 to convert the volume into micro mol.

5) The answer from step 4 in µmol was divided by 0. 1 which was the amount of enzyme used.

Example for Ethanol:

1) 0. 851 ÷ 6220 = 1. 37 x

2) 1. 37 x10-4 × 0. 003 = 4. 10 x10-7

3) 4. 10 x10-7 × 106 = 0. 41

4) 0. 41 ÷ 0. 1 = 4. 1µmol min-1 ml-1

The same method was used for the velocity calculation of others. The results have been tabulated above.

Ethanol:

Table 4

Ethanol

Gradient

Enzyme co-efficient (l/mol/cm)

Cuvette (L)

Convert to micromoles (µmol)

Enzyme used (µmol min-1 ml

0. 851

6220

0. 003

1000000

0. 1

0. 502

6220

0. 003

1000000

0. 1

0. 331

6220

0. 003

1000000

0. 1

0. 356

6220

0. 003

1000000

0. 1

0. 192

6220

0. 003

1000000

0. 1

Table 5

Substrate Concentration (M)

Velocity (V)

0. 080

4. 105

0. 040

2. 421

0. 020

1. 596

0. 010

1. 717

0. 005

0. 926

Propan-1-ol:

Table 6

Propan-1-ol

Gradient

Enzyme co-efficient (l/mol/cm)

Cuvette (L)

Convert to micromoles

enzyme used

0. 174

6220

0. 003

1000000

0. 1

0. 165

6220

0. 003

1000000

0. 1

0. 116

6220

0. 003

1000000

0. 1

0. 097

6220

0. 003

1000000

0. 1

0. 080

6220

0. 003

1000000

0. 1

Table 7

Substrate Concentration (M)

Velocity (V)

0. 080

0. 839

0. 040

0. 796

0. 020

0. 559

0. 010

0. 468

0. 005

0. 386

Butan-1-ol:

Table 8

Butan-1-ol

Gradient

Enzyme co-efficient (l/mol/cm)

Cuvette (L)

Convert to micromoles

enzyme used

0. 235

6220

0. 003

1000000

0. 1

0. 159

6220

0. 003

1000000

0. 1

0. 144

6220

0. 003

1000000

0. 1

0. 093

6220

0. 003

1000000

0. 1

0. 069

6220

0. 003

1000000

0. 1

Table 9

Substrate Concentration (M)

Velocity (V)

0. 080

1. 133

0. 040

0. 767

0. 020

0. 695

0. 010

0. 449

0. 005

0. 333

The Michaelis Menten graphs are attached, fig 2. 5, 2. 6 and 2. 7.

Lineweaver Burk plots

. Since the velocity had already been calculated from the gradient values of the previous absorbance plots, they could be used in plotting the above mentioned plots.

The Graphs of were plotted by dividing the values of velocity and substrate concentrations both by 1 Now that all the info was here, it was possible to plot the Lineweaver Burk plots.

Ethanol:

Table 10

Ethanol

Substrate Concentration (M)

Velocity (V)

1/S

1/V

0. 080

4. 105

12. 500

0. 244

0. 040

2. 421

25. 000

0. 413

0. 020

1. 596

50. 000

0. 627

0. 010

1. 717

100. 000

0. 582

0. 005

0. 926

200. 000

1. 080

Fig 2. 8

Propan-1-ol:

Table 11

Propan-1-ol

Substrate Concentration (M)

Velocity (V)

1/[S]

1/V

0. 080

0. 008

12. 500

125. 000

0. 040

0. 008

25. 000

125. 000

0. 020

0. 006

50. 000

166. 667

0. 010

0. 005

100. 000

200. 000

0. 005

0. 004

200. 000

250. 000

Fig 2. 9

Butan-1-ol:

Table12

Butan-1-ol

Substrate Concentration (M)

Velocity (V)

1/S

1/V

0. 080

1. 133

12. 500

0. 883

0. 040

0. 767

25. 000

1. 304

0. 020

0. 695

50. 000

1. 439

0. 010

0. 449

100. 000

2. 227

0. 005

0. 333

200. 000

3. 003

Fig 3. 0

Eadie Hofstee

The final set of graphs that needed to be plotted were the Eadie Hofstee plots. These are plots of V/[S] against velocity. All graphs for each alcohol had a negative gradient because of the inverse relationship between the V/S and velocity.

Ethanol:

Table 13

Ethanol

Substrate Concentration (M)

Velocity (V)

V/S

0. 080

4. 105

51. 313

0. 040

2. 421

60. 525

0. 020

1. 596

79. 800

0. 010

1. 717

171. 700

0. 005

0. 926

185. 200

Fig 3. 1

Propan-1-ol:

Table 14

Propan-1-ol

Substrate Concentration (M)

Velocity (V)

V/S

0. 080

0. 839

10. 488

0. 040

0. 796

19. 900

0. 020

0. 559

27. 950

0. 010

0. 468

46. 800

0. 005

0. 386

77. 200

Fig 3. 2

Butan-1-ol:

Table 15

Butan-1-ol

Substrate Concentration (M)

Velocity (V)

V/S

0. 080

1. 133

14. 163

0. 040

0. 767

19. 175

0. 020

0. 695

34. 750

0. 010

0. 449

44. 900

0. 005

0. 333

66. 600

Fig 3. 3

Kcat Calculations

1) Step one is: 0. 1÷1000= 0. 001g/ml

2) The molecular weight of the enzyme was 150, 000g/mol

3) The 0. 001 g/ml is divided by 150, 0000. 001÷150000= 6. 67×mol/ml

4) Convert mol to µmol by 6. 67×mol/ml × = 6. 67µmol/ml

5) Kcat= Vmax÷[E]t

Kcat = 3. 48 ÷ 6. 67 = 5217min-1

1/60 5217 = 86. 95 s-1

Summary table

Vmax, Km and Kcat values for each substrate and each analytical method used have been summarized in the tables below.

Table 16

Ethanol

Propan-1-ol

Butan-1-ol

Velocity

Vmax (V)

4. 2

0. 85

1. 145

Km (M)

0. 031

0. 07

0. 015

Kcat

Lineweaver Burk

Vmax (V)

3. 48

0. 82

1. 076

Km (M)

0. 01

0. 01

0. 011

Kcat

86. 95

20. 48

26. 88

Eadie Hofstee

Vmax (V)

4. 96

0. 913

1. 25

Km (M)

0. 026

0. 01

0. 016

Kcat

123. 93

22. 81

31. 23

Lineweaver Burk

For the Lineweaver Burk method the Vmax and Km values were worked out using the following method:

Example for Propan-1-ol:

Equation of the linear line: y= 0. 007x + 1. 216

Step 1

The value 1. 216 has been taken from the linear line equation, which in this case is C in the equation.

Step 2

Km = Slope Vmax

The value for the slop was taken from the linear line equation which was the gradient. The Vmax in the above equation has already been calculated in step 1.

Eadie Hofstee

Example for Butan-1-ol:

Linear line equation: y=-62. 20x + 77. 92

Y= 0

To work out the Vmax we want “ x” to be the subject therefore:

To work out the Km the following equation was used:

The -62. 20 value was taken from the gradient of the linear line equation. This value of gradient was then by -1 because this value was part of the Eadie Hofstee equation.

Discussion

Only the first 4 plots were used in making the Absorbance vs. Time because using all the absorbance values would give a curvy orientation to the graph. The reason for this is that the enzyme sites were occupied by the substrates because of which there were no more enzymes sites where the substrate could bind. This is an indication of the Vmax meaning the all the catalytic sites have been occupied and the enzyme has become saturated with the substrate.