

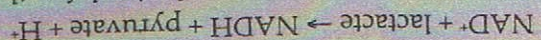
Reinate
Nr. 2
0941217

Biophysical Chemistry

Week 11 Problems

To be handed in by Thursday 5th June 2014, 15:00

1. The enzyme lactate dehydrogenase catalyses the following reaction:



The following data were obtained for the effect of pyruvate (a product) on the reaction:

[Pyruvate]	[Lactate]	0 μM	40 μM	80 μM
1.5	1.88 katal/kg	1.05	0.73	
2.0	2.36	1.34	0.94	
3.0	3.1	1.88	1.34	
10.0 mM	5.81	4.19	3.27	

- (a) Calculate K_m and V_{max} for the different conditions and determine which type of inhibition is being observed.

(b) Calculate the K_i value.

- (c) Dehydrogenases normally follow a 'ternary complex' mechanism; draw the corresponding Cleland diagram.

2. A hexosaminidase from *Caenorhabditis elegans* was expressed in *Pichia pastoris* and assayed using *p*-nitrophenyl- β -N-acetylglucosaminide (PNP-GlcNAc). In a series of tests, the activity with different concentrations of PNP-GlcNAc was examined in the presence of different concentrations of an inhibitor, N-acetylcastanospermine (NACS). Using the data below, calculate the K_m for PNP-GlcNAc and the K_i for NACS and determine whether it is competitive inhibition or not.

PNP-GlcNAc concentrations		Absorption at different NACS concentrations				
PNP-GlcNAc [mM]	0 μM	6.25 μM	9.375 μM	12.5 μM	25 μM	
0.5	0.152	0.085	0.073	0.050	0.038	
1	0.212	0.121	0.106	0.093	0.053	
1.5	0.261	0.171	0.154	0.135	0.082	
2	0.300	0.219	0.194	0.166	0.104	
2.5	0.325	0.272	0.245	0.233	0.138	

NB: Due to the low absorbance values in the presence of inhibitor, the best results with the K_i plot will be obtained when not including the data obtained with 0.5 mM PNP-GlcNAc.

pNP-GlcNAc					
Absorption at different NACS concentrations					
[mM]	0 μM	6,25 μM	9,375 μM	12,5 μM	25 μM
0,5	0,152	0,085	0,073	0,05	0,038
1	0,212	0,121	0,106	0,093	0,053
1,5	0,261	0,171	0,154	0,135	0,082
2	0,3	0,219	0,194	0,166	0,104
2,5	0,325	0,272	0,245	0,233	0,138

1/S	0 μM	6,25 μM	9,375 μM	12,5 μM	25 μM
2	6,57894737	11,7647059	13,6986301	20	26,3157895
1	4,71698113	8,26446281	9,43396226	10,7526882	18,8679245
0,66666667	3,83141762	5,84795322	6,49350649	7,40740741	12,195122
0,5	3,33333333	4,56621005	5,15463918	6,02409639	9,61538462
0,4	3,07692308	3,67647059	4,08163265	4,29184549	7,24637681

$x\text{-intercept} = -1/km$
 $km = 1/x\text{-int.}$
 $0 = 2,1713x + 2,3244$
 $x = -1,07051075$
 $km = 0,93413354 \text{ mM}$

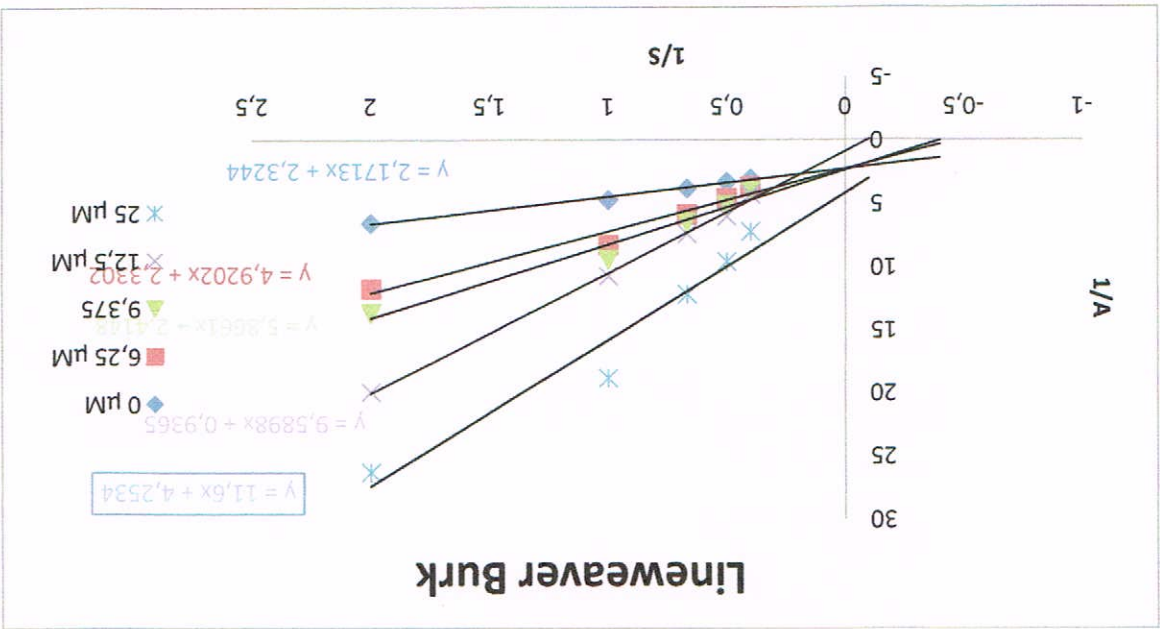
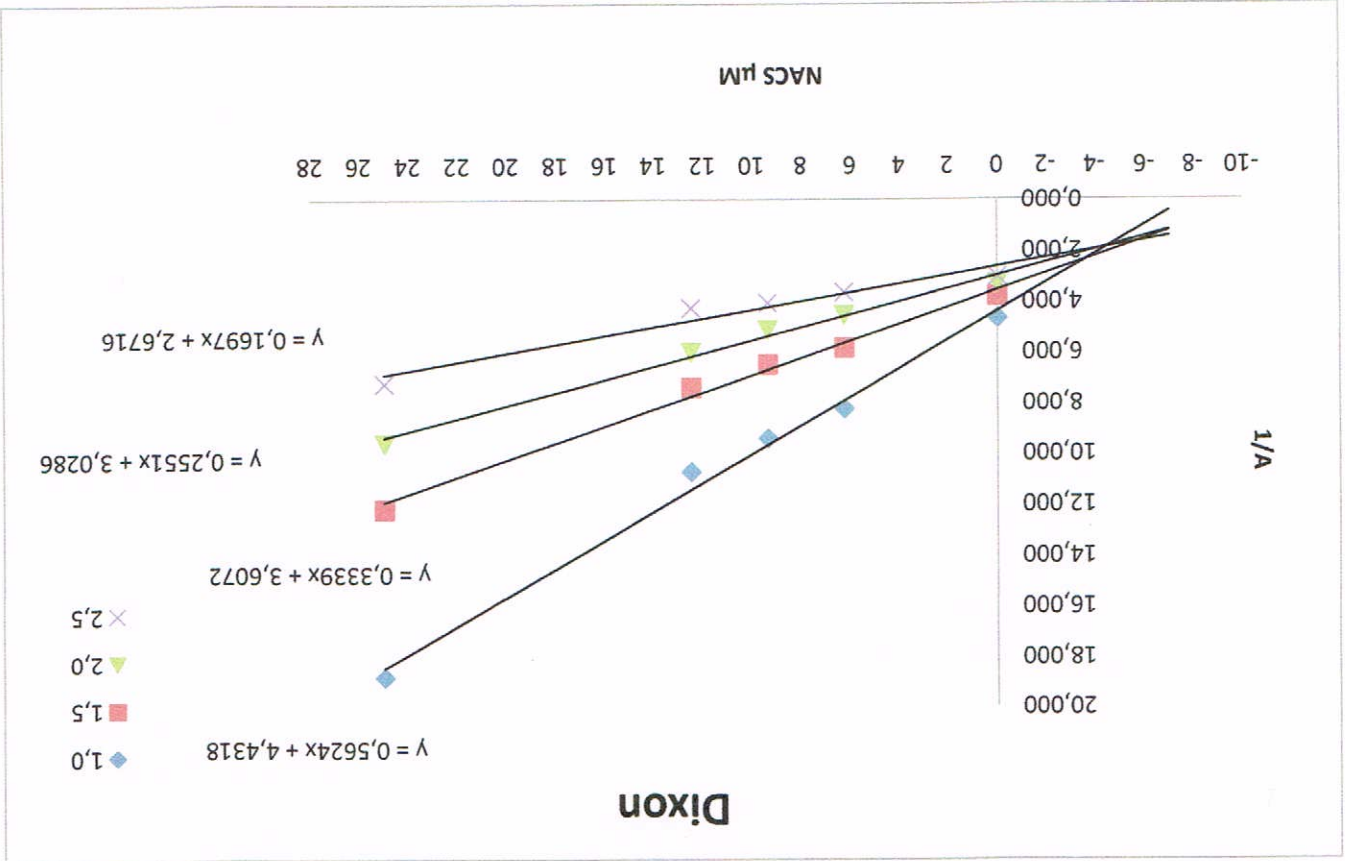
1/S	0 μM	6,25 μM	9,375 μM	12,5 μM	25 μM
2	6,57894737	11,7647059	13,6986301	20	26,3157895
1	4,71698113	8,26446281	9,43396226	10,7526882	18,8679245
1,5	3,83141762	5,84795322	6,49350649	7,40740741	12,195122
2	3,33333333	4,56621005	5,15463918	6,02409639	9,61538462
2,5	3,07692308	3,67647059	4,08163265	4,29184549	7,24637681

Dixon-Plot

pNP-GlcNAc [mM]					
1/Abs					
NACS [μM]	0	6,25	9,375	12,5	25
0	6,579	4,717	3,831	3,333	3,077
6,25	11,765	8,264	5,848	4,566	3,676
9,375	13,699	9,434	6,494	5,155	4,082
12,5	20,000	10,753	7,407	6,024	4,292
25	26,316	18,868	12,195	9,615	7,246

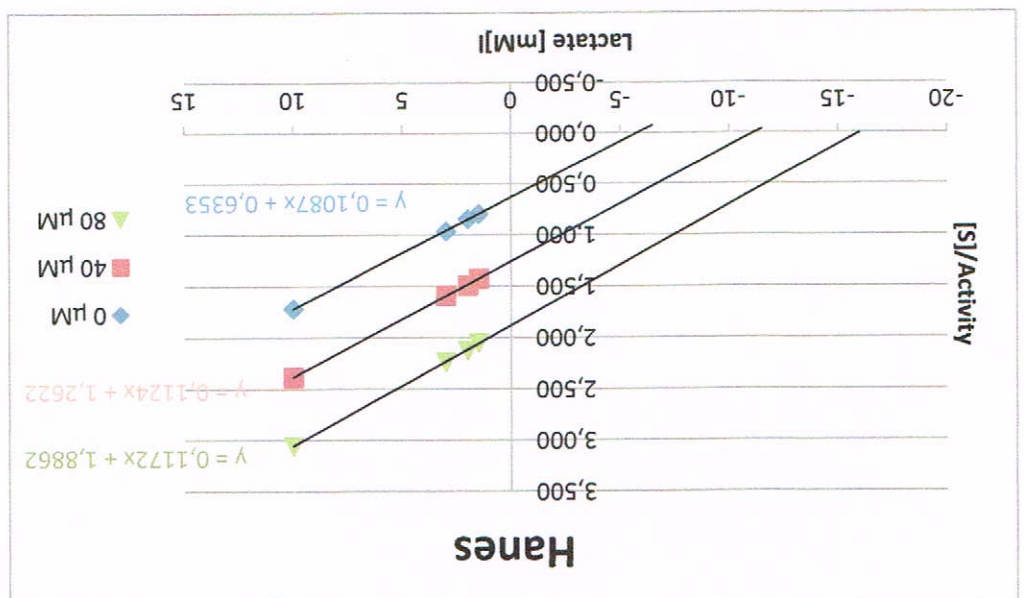
competitive inhibition because $1/kM$ is affected while V_{max} is not

$0,1697x + 2,6716 = 0,2551x + 3,0286$
 Estimated K_i value by optical method: -5 mM
 $K_i = -4,180 \text{ mM}$



Pyruvate [μM]	Lactate [mM]			
	1,5	2	3	3,27
0	1,88	2,36	3,1	5,81
40	1,05	1,34	1,88	4,19
80	0,73	0,94	1,34	3,27

Pyruvate/ Lactat	[S]/Aktivität			
	0 μM	40 μM	80 μM	
	0,798	1,429	2,055	3,058
	0,847	1,493	2,128	2,239
	0,968	1,596		
	1,721	2,387		

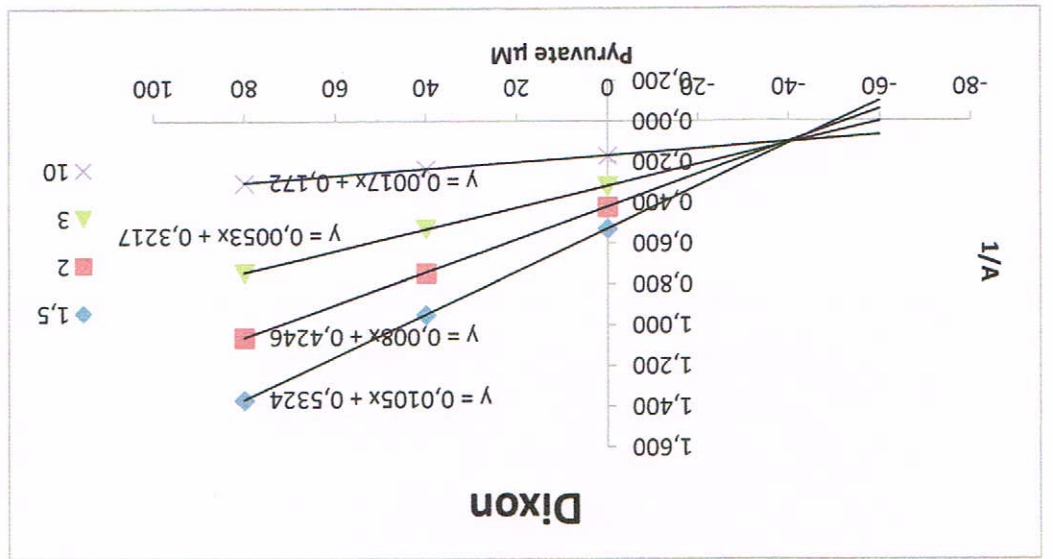


Pyruvat/Lactat	gradient = 1/	Vmax = 1/ gradient
0 μM	0,1087	9,20 $\text{mol s}^{-1} \text{kg}^{-1}$
40 μM	0,1124	8,90 $\text{mol s}^{-1} \text{kg}^{-1}$
80 μM	0,1172	8,53 $\text{mol s}^{-1} \text{kg}^{-1}$

Pyruvat/Lactat	x-intercept = Km = d/k
0 μM	0,635/0,108
40 μM	1,262/0,112
80 μM	1,886/0,117

Conclusion: Pyruvat acts as a competitive inhibitor (Km \downarrow but Vmax nearly constant) towards Lactate

Pyruvate/ Lactat	1/Aktivität			
	1,5	2	3	10
0 µM	0,532	0,424	0,323	0,172
40 µM	0,952	0,746	0,532	0,239
80 µM	1,370	1,064	0,746	0,306



K_I
 $0,0017x + 0,172 = 0,0053x + 0,3217$
 $x = 41,6 \mu M$
 $K_I = 41,6 \mu M$

Cleland - Diagramme lactate dehydrogenase

