<u>Assignment 1:</u>

4 ml of enzyme preparation isolated from the cytoplasm of beef heart contains 128 mg malate dehydrogenase protein of the relative molecular mass 52 000.
L-Malate 1.5 x 10^{-2} mol x l^{-1} was employed as a substrate in the enzyme reaction. The value of K_m for the substrate is 9.9 x 10^{-4} mol x l^{-1}. An aliquot part of the original enzyme solution was diluted by a factor 10^{-3} (1000-fold). Under standard conditions (zero-order kinetics) 0.01 ml of the diluted enzyme preparation was able to transform 0.1 μmol substrate per minute.

a) Calculate the specific activity of malate dehydrogenase as μkat x mg^{-1} protein.

b) Estimate the maximal velocity ot the enzyme reaction as μkat x mg^{-1} protein.

c) Calculate the mole activity of malate dehydrogenase as kat x mol^{-1}.

d) Calculate the actual reaction rate for the given substrate concentration as % of the maximal velocity, assuming comparable conditions and taking the maximal velocity in the excess of substrate as 100 % (zero-order kinetics).

e) Calculate the concentration of malate dehydrogenase in the original undiluted enzyme solution as kat x l^{-1}.

f) Calculate at which substrate concentration expressed as mmol x l^{-1} the initial velocity of the enzyme reaction (v_o) would be equal to 80% of the maximal velocity in the excess of substrate under comparable conditions (zero-order kinetics).

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a) Calculate the specific activity of malate dehydrogenase as μkat x mg^{-1} protein.

0.01 ml …………… 0.1×10^3 μmol x min^{-1}
1.00 ml …………... 10.0×10^3 μmol x min^{-1}
4.00 ml …………… 40.0×10^3 μmol x min^{-1}

$$\frac{40.0 \times 10^3}{60.0} = 666.667\ \mu mol \times s^{-1} = 666.667\ \mu kat$$

666.667 μkat ………………….128 mg protein
x …………………. 1 mg protein

$$x = \frac{666.667 \times 1}{128} = 5.208\ \mu kat \times mg^{-1}\ protein$$

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b) Estimate the maximal velocity ot the enzyme reaction as μkat x mg^{-1} protein.

$$v = \frac{V}{1 + \frac{K_m}{S}}$$

$$V = v \left(1 + \frac{K_m}{S} \right)$$

$$V = 5.208 \left(1 + \frac{9.9 \times 10^{-4}}{1.5 \times 10^{-2}} \right) = 5.208\ (1 + 0.066)$$

V = 5.552 μkat x mg^{-1} protein

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c) Calculate the mole activity of malate dehydrogenase as kat x mol^{-1}.

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1.00 ml …………... 10.0×10^3 µmol x min^{-1}
4.00 ml …………… 40.0×10^3 µmol x min^{-1}

$$\frac{40.0 \times 10^3}{60.0} = 666.667\ \mu mol \times s^{-1} = 666.667\ \mu kat$$

128 mg = ? mol

$$c_n = \frac{c_m}{M_r} = \frac{0.128}{5.2 \times 10^4} = 0.024615 \times 10^{-4} = 2.462 \times 10^{-6}\ mol$$

666.667 µkat …………….. 2.462×10^{-6} mol
x ……………... 1 mol

$$x = \frac{666.667 \times 1}{2.462 \times 10^{-6}} = 270.783 \times 10^6\ \mu kat \times mol^{-1} = 270.783\ kat \times mol^{-1}$$

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d) Calculate the actual reaction rate for the given substrate concentration as % of the maximal velocity, assuming comparable conditions and taking the maximal velocity in the excess of substrate as 100 % (zero-order kinetics).

$$v = \frac{V}{1 + \frac{K_m}{S}}$$

$$v = \frac{100}{1 + \frac{9.9 \times 10^{-4}}{1.5 \times 10^{-2}}} = \frac{100}{1 + 0.066} = 93.8$$

v = 93.8 %

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e) Calculate the concentration of malate dehydrogenase in the original undiluted enzyme solution as kat x l^{-1}.

0.01 ml …………… 0.1×10^3 µmol x min^{-1}
1.00 ml …………... 10.0×10^3 µmol x min^{-1}
4.00 ml …………… 40.0×10^3 µmol x min^{-1}

$$\frac{40.0 \times 10^3}{60.0} = 666.667\ \mu mol \times s^{-1} = 666.667\ \mu kat$$

666.667 µkat ………………. 4 ml
x …………….. 1000 ml

$$x = \frac{666.667 \times 1000}{4} = 166666.75\ \mu kat \times l^{-1} = 0.167\ kat \times l^{-1}$$

x = 0.167 kat x l^{-1}

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f) Calculate at which substrate concentration expressed as mmol x l^{-1} the initial velocity of the enzyme reaction (v_o) would be equal to 80% of the maximal velocity in the excess of substrate under comparable conditions (zero-order kinetics).

$$v = \frac{V}{1 + \frac{K_m}{S}}$$

V = 100 %
v = 80 %
S = ?

$$80 = \frac{100}{1 + \frac{9.9 \times 10^{-4}}{S}}$$

$$\frac{1}{80} = \frac{1 + \frac{9.9 \times 10^{-4}}{S}}{100}$$

$$\frac{100}{80} = 1 + \frac{9.9 \times 10^{-4}}{S}$$

$$\frac{9.9 \times 10^{-4}}{S} = 0.25$$

$$0.25\ S = 9.9 \times 10^{-4}$$

$$S = 3.96 \times 10^{-3}\ \text{mol x l}^{-1} = 3.96\ \text{mmol x l}^{-1}$$

malate dehydrogenase	**cytoplasm**		**mitochondria**	
substrate	**malate**	**oxaloacetate**	**malate**	**oxaloacetate**
specific activity **$\mu kat \cdot mg^{-1}$ protein**	**5.208**	**18.75**	**8.854**	**15.10**
mole activity **$kat \cdot mol^{-1}$**	**270.8**	**975.0**	**548.9**	**936.3**
enzyme concentration **$kat \cdot l^{-1}$**	**0.167**	**0.600**	**0.283**	**0.483**
K_m **$mol \cdot l^{-1}$**	**9.9×10^{-4}**	**4×10^{-5}**	**5.4×10^{-4}**	**5.1×10^{-5}**

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