

<i>THE MALATE DEHYDROGENASE LABORATORIES</i>	Page
Introduction to Malate Dehydrogenase	2
Examining MDH	5
The Structure and Absorption Spectrum of NADH	7
Reaction Rate Measurements: Initial Rate Measurements: overview of experimental design and data analysis	9
Experiment	
Malate Dehydrogenase Activity Measurements	11
<i>Quantitative Analysis of the Data</i>	
Experiment	
The Effects of Temperature on Reaction Rate Measurements: Activation Energies	12
Malate Dehydrogenase Activity Measurements	
<i>Quantitative Analysis of the Data</i>	13
Reproducibility, Error analysis and the basics of data presentation	16
Using the Spectrophotometer	18

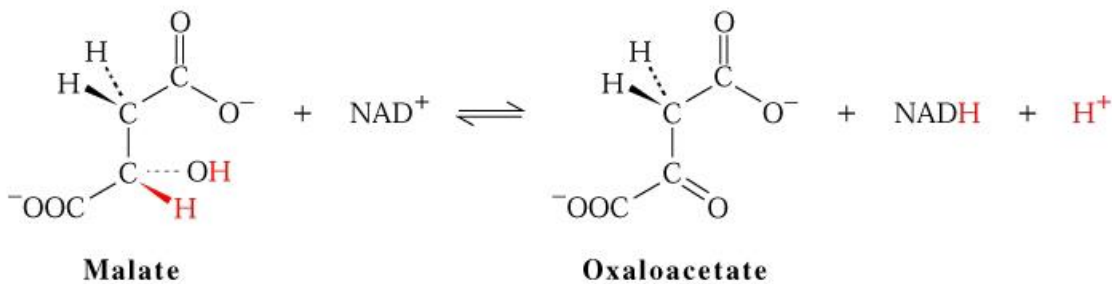
The Malate Dehydrogenase Laboratories

Introduction to Malate Dehydrogenase

Malate Dehydrogenases catalyze the reaction:

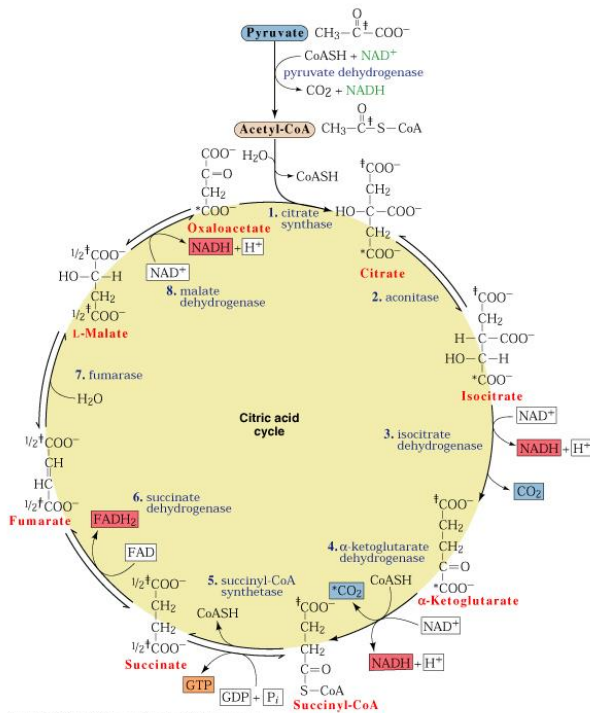


Involving a simple hydride transfer from the 2 position of Malate



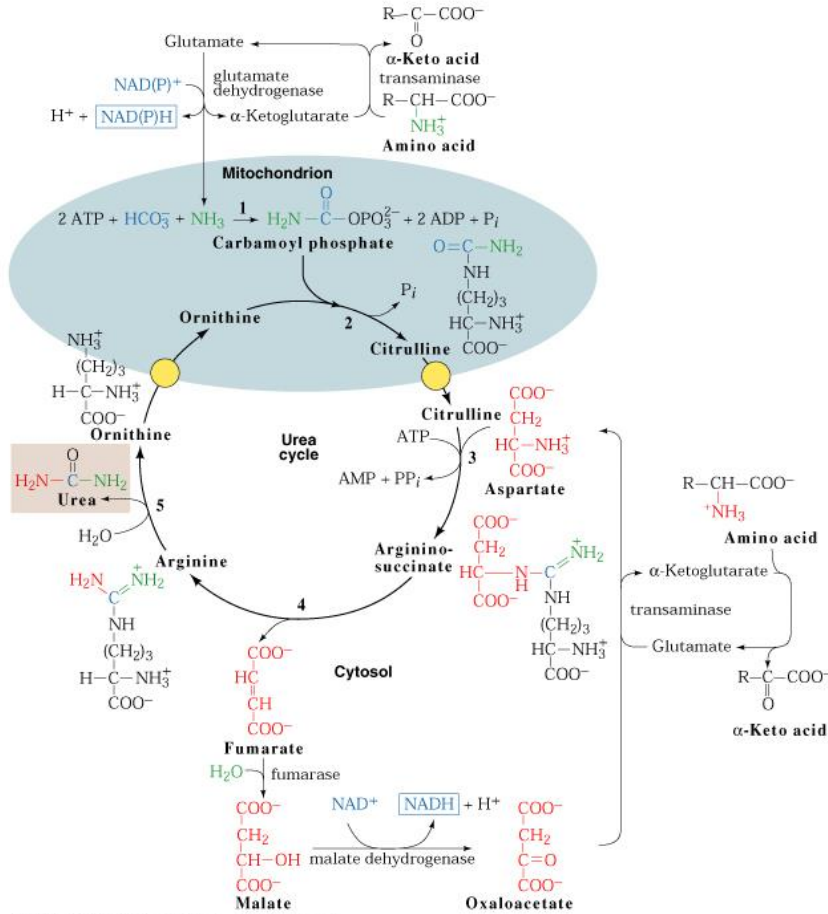
Copyright 1999 John Wiley and Sons, Inc. All rights reserved.

to the nicotinamide ring of NAD^+ to give NADH . During the process a proton is also released to the solvent. This reaction plays a number of important roles in metabolism, illustrated by a reaction in the Tricarboxylic acid cycle:



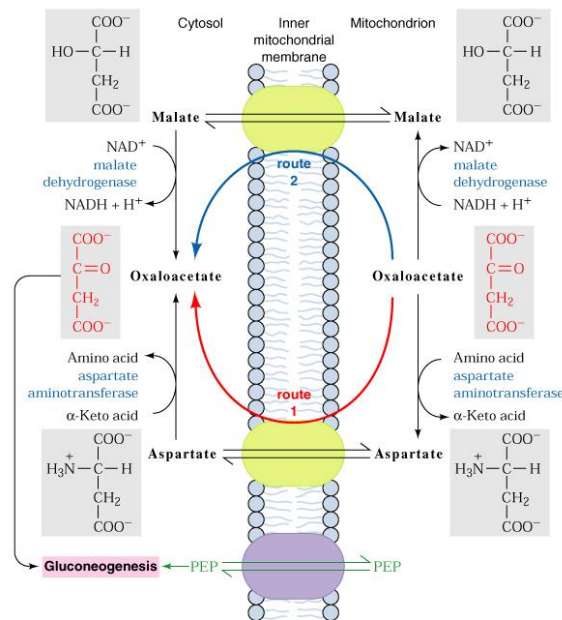
Copyright 1999 John Wiley and Sons, Inc. All rights reserved.

A reaction critical to the Urea Cycle:



Copyright 1999 John Wiley and Sons, Inc. All rights reserved.

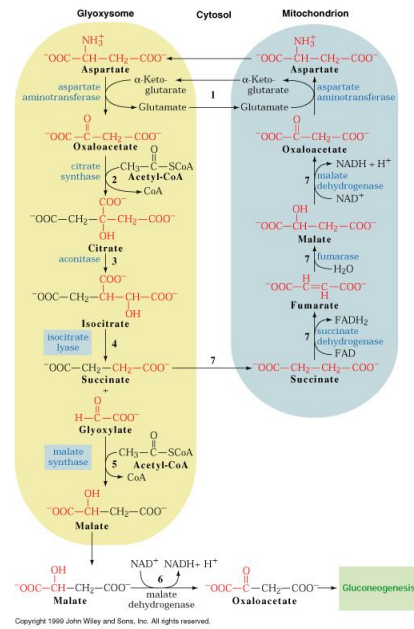
a reaction playing a role in the shuttling of reducing equivalents into mitochondria:



Copyright : Jessic.

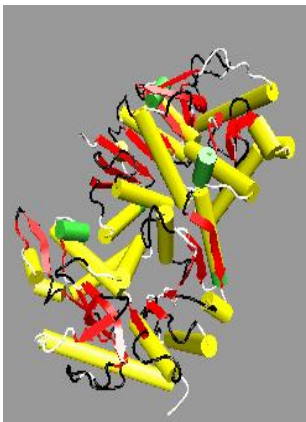
Copyright 1999 John Wiley and Sons, Inc. All rights reserved.

and in plants a reaction in the Glyoxasome:

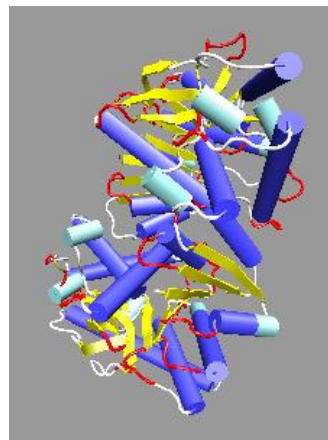


It is clear that there must exist Malate Dehydrogenase in at least two different locations within the cell and in fact there are two distinct isoenzymes, a cytoplasmic MDH [cMDH] and a mitochondrial MDH [mMDH] in higher eukaryotes which have different amino acid sequences and somewhat different three dimensional structures:

cMDH



mMDH



Each is a dimer [as shown above], and each subunit contains two domains, with a classic dinucleotide binding domain [to the left] and a malate binding domain [to the right]. In mitochondria MDH is thought to form loose multienzyme complexes with several other enzymes sharing substrates, in particular Aspartate AminoTransferases which catalyze the transamination of Glutamate and Oxaloacetate to give Aspartate and 2-Oxoglutarate, a key reaction in the Glyoxylate Cycle and Gluconeogenesis:

Examining Malate Dehydrogenase

The sequence of porcine Malate Dehydrogenase is easily obtained by going to the protein data base and searching for the three dimensional structure of Malate Dehydrogenase [<http://www.rcsb.org/pdb/>]. From the structure file, which you can download to examine later, you can get the amino acid sequence in FASTA format [a format used by most data base search engines].

Copy the sequence and enter it into BLAST [<http://www.ncbi.nlm.nih.gov/BLAST/>] and see how many similar sequences are found in the non-repetitive protein data base.

To see just a little of the diversity of physiological activities of these Malate Dehydrogenase like proteins randomly investigate the putative roles of some of these proteins using the links provided in the BLAST search results. You will find proteins from almost every type of life form [except viruses] and from a variety of cellular compartments.

You can also obtain a wealth of information about both how many malate dehydrogenases, and the types of activity that they are involved with by searching PUBMED [<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi>] using one of its options [the drop down menus on the left of the screen-where it says "search"] to search the protein data base. Enter the search words "Malate Dehydrogenase" and you will come up with a wide variety of Malate Dehydrogenase like proteins. Likewise, PUBMED is a valuable source of literature citations for Malate Dehydrogenase. On the subject of finding background information about a given protein, you can usually find a variety of relevant references by reading the introduction of a paper about Malate Dehydrogenase. The introduction to a well written paper will usually give you background information as to what the protein does, where it is found, often molecular characteristics of the protein and sometimes information about kinetic properties etc. For the papers that you will find useful in this sequence of laboratories, ones about structure or kinetic/regulatory properties are likely to be the most useful.

To get an idea of involvement of Malate Dehydrogenase in human disease you can search OMIN:

"OMIM, Online Mendelian Inheritance in Man. This database is a catalog of human genes and genetic disorders authored and edited by Dr. Victor A. McKusick and his colleagues at Johns Hopkins and elsewhere, and developed for the World Wide Web by NCBI, the National Center for Biotechnology Information. The database contains textual information and references. It also contains copious links to MEDLINE and sequence records in the Entrez system, and links to additional related resources at NCBI and elsewhere."

Finally, you can easily examine the three dimensional structure of Malate Dehydrogenase [or of course any other protein whose three dimensional structure has been determined] using the tools at the Protein Data Base[<http://www.rcsb.org/pdb/>]

Table 1: Non-Polar Amino Acids: Chemical structures shown at pH 1.0:

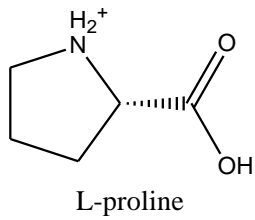
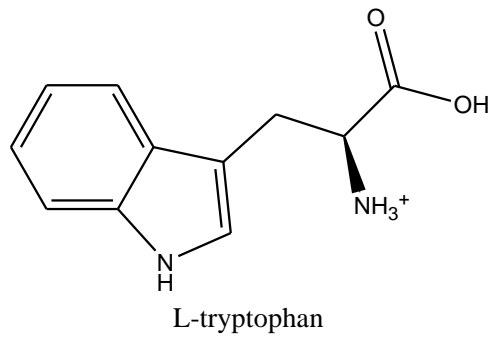
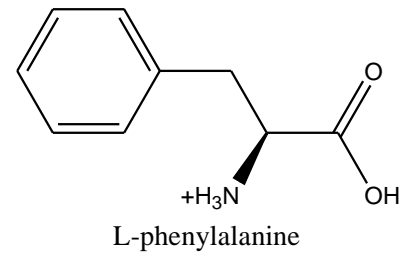
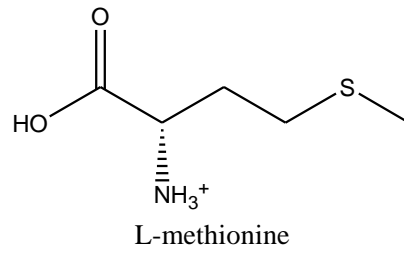
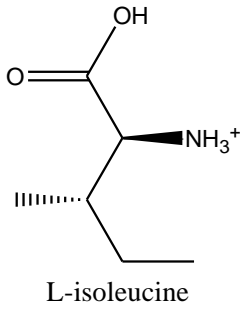
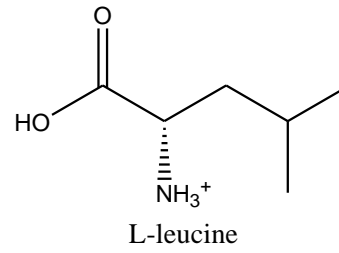
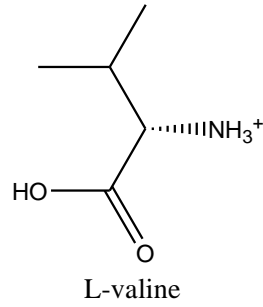
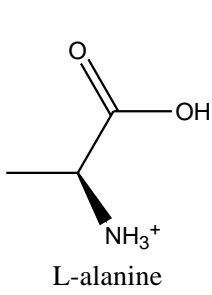
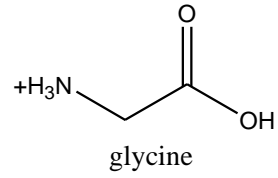
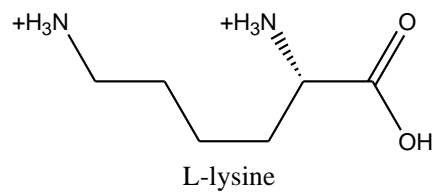
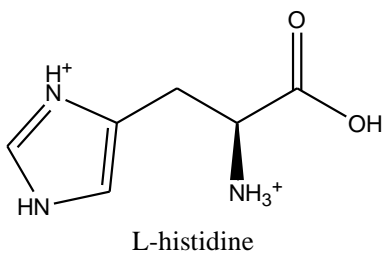
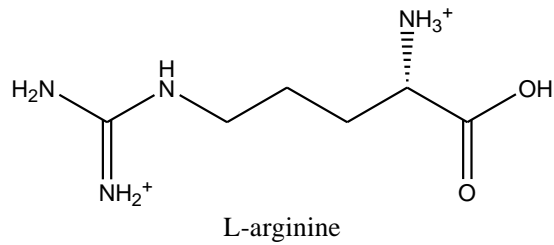
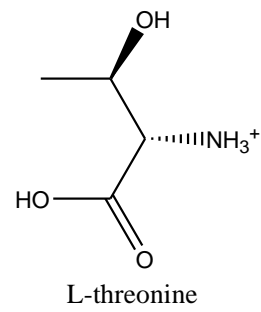
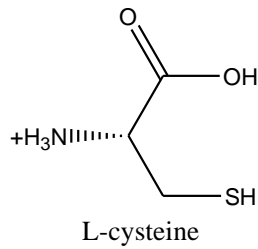
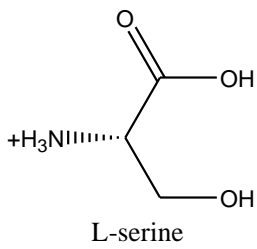
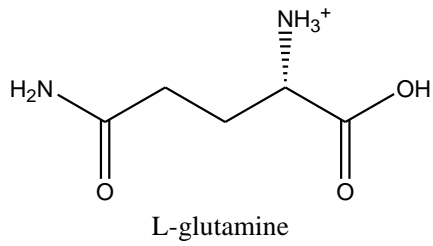
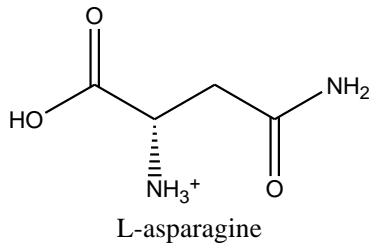
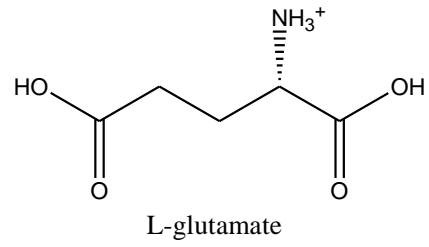
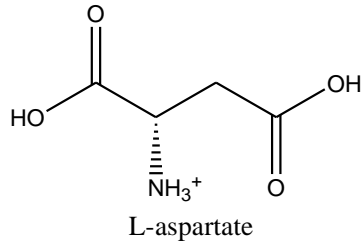
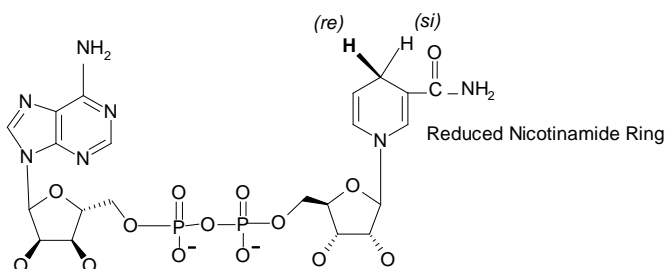


Table 2: Polar Amino Acids



Structure and Absorption Spectrum of NADH:

There are two chromophores in the molecule, the reduced nicotinamide ring [labeled] and the adenine ring. Each has a distinct absorption spectrum [plot of Absorbance versus Wavelength] as you will see in the first part of the experiment and the Extinction Coefficient of either absorption band can be used to quantitate the concentration of NADH in solution. In the experiment here you will utilize the calibration of the concentration of the NADH solution that you made up, at 260nm, to determine the extinction coefficient at 340nm.



What Do You Need to Measure?

To determine the absorption spectrum of NADH you need to first measure the absorption of the buffer that you plan to use- in this case 50mM Phosphate Buffer at pH 8.0, and then add a known volume of the NADH solution that you are trying to determine the concentration of.

If you have no idea of the amount of NADH to add, what do you do?

If you are to make a meaningful measurement what else would you want to know?

Quantitative Analysis of the Data

Lets consider that in an experiment similar to that described above the following data was obtained:

Having made up a solution of NADH that was, according to weight, 2mM you measure the absorbance at 260nm by adding 25 μ L to 1mL of buffer and obtain an absorbance of 0.660

The concentration of the NADH solution is obtained using the millimolar absorbtivity of NADH at 260nm of 14.4cm⁻¹.

Thus the concentration in the cuvette that gave an absorbance of 0.66 is:

$$0.66/14.4 = 0.0458\text{mM}$$

This was obtained by diluting the original NADH solution 25 μL into a total volume of 1025 μL , a dilution factor of $1025/25 = 41$

Thus the concentration of the original NADH slution was $0.0458 \times 41 = 1.879\text{mM}$

How do you now calculate the millimolar absorptivity of NADH at 340nm?

Laboratory

Reaction Rate Measurements: Initial Rate Measurements

Overview of the Experiments in this laboratory

Background for the Measurement of Initial Rates of Enzyme Catalyzed Reactions

A fundamental property of an enzyme is to enhance the rate of a chemical reaction. It does this by lowering the energy level of the transition state and hence lowering the barrier between substrates and products at any given temperature. The result is that more reactants reach the transition state energy level and hence can proceed to products in the presence of the enzyme than occurs in the absence of the enzyme at a given temperature. The lowered energy of the transition state results from the physical and chemical nature of the enzyme-transition state complex. The activation energy of an enzyme catalyzed reaction is thus an indicator of this complex and is characteristic for a given state of an enzyme. The experiment links two important concepts: the measurement of the rates of a reaction- in the case of an enzyme catalyzed reaction, the "initial rate", often represented as v_0 , and the effects of temperature on the rate of the reaction, which allows the energy of activation to be quantitated for any reaction, whether it be chemical or enzymatic. Each of these facets of the experiment are first developed from a theoretical standpoint and then from an experimental design standpoint together with the rationale for the overall design of the experiments.

Introduction to Making Measurements of the Initial Rate of an Enzyme Catalyzed Reaction

Overview:

The measurement of the rate of a reaction depends upon being able to estimate either the amount of substrate [A] present or the amount of product [P] present as a function of time and the rate [often referred to as the velocity, v] of the reaction is simply:

$$v = -d[A]/dt = d[P]/dt \qquad \text{Equation 12.1}$$

with the rate of the reaction being proportional to the concentration of the reactant, A, where

$v = k[A]$, where k is the rate constant of the reaction. Measurement of the velocity as a function of the concentration of A allows the rate constant, k to be determined.

Enzyme catalyzed reactions are a little more complex and derivation of the Michaelis-Menten equation [equation 12.25] is based on the ability to measure the initial velocity, v_0 , defined as the velocity of the reaction immediately after the enzyme steady state has

been achieved. Since the determination of the properties of an enzyme depend upon various applications of the Michaelis-Menten equation it is critically important that the initial velocity of an enzyme catalyzed reaction is accurately measured. The following experiment illustrates this point and will familiarize you with the types of calculations that are involved in measuring the rates of enzyme catalyzed reactions.

Determining the Initial Rate of the Malate Dehydrogenase Catalyzed Reaction

Overview of the Experiment:

The purpose of this experiment is to determine the initial rate of the malate dehydrogenase catalyzed reaction and to demonstrate that over a limited range of enzyme concentrations it is a linear function of the enzyme concentration. This linearity is predicted only if the rate measurements are in fact "initial rates". Furthermore, once it is established that initial rates are in fact being measured we will go on to examine the effects of temperature on the rate of the reaction. This will allow us to calculate the activation energy of the reaction.

In the case of an enzyme such as Malate dehydrogenase, which catalyzes the reaction:



where two substrates are involved, NAD^+ and Malate in the forward direction, NADH and Oxaloacetate in the "reverse" of the reaction there is another consideration: what concentration of the two substrates to use. The answer in the case in point is that we want a reference set of conditions where the rate is strictly proportional to the enzyme concentration. We will refer to those conditions as "standard assay conditions" and here they consist of 0.02M Phosphate Buffer, pH 8.0 containing 1mM Oxaloacetate and 0.1mM NADH . These conditions, will be used both here and in the purification of Malate Dehydrogenase described in later experiments since as you will find they are conditions where the rate is directly proportional to the enzyme concentration. They are the standard conditions used to describe the "Specific Activity" of Malate Dehydrogenase in the various experiments described in this book.

You will have noticed that in all of the discussion of Malate Dehydrogenase the utilization of NADH has been used in the assay. Why not use the experimentally simpler $\text{NAD}^+ \rightarrow \text{NADH}$ direction of the reaction?

The answer is actually quite simple if you remember that a reaction can reach an equilibrium position, and in the case of MDH the equilibrium position significantly favors Malate/ NAD^+ and as a result the oxidation of Malate goes rapidly to equilibrium making it very difficult to determine an initial rate in the $\text{NAD}^+ \rightarrow \text{NADH}$ direction.

Designing Your Own Experiment: Experiment 1.2

Measuring the Initial Rate and Specific Activity of Malate Dehydrogenase in the "Reverse" Reaction

You should conduct a similar experiment using a "standard assay" of the reverse reaction. Of Malate Dehydrogenase. This standard assay should consist of 0.05M Phosphate Buffer, pH 8.0 containing 100 μ M NADH, and 1mM Oxaloacetate.

You are provided with the following solutions:

0.05M Phosphate Buffer, pH 8.0

6.2mM NADH in H₂O

62mM Oxaloacetate in H₂O

0.05mg/mL Malate dehydrogenase in 0.05M Phosphate at pH 8.0

Points to Think About:

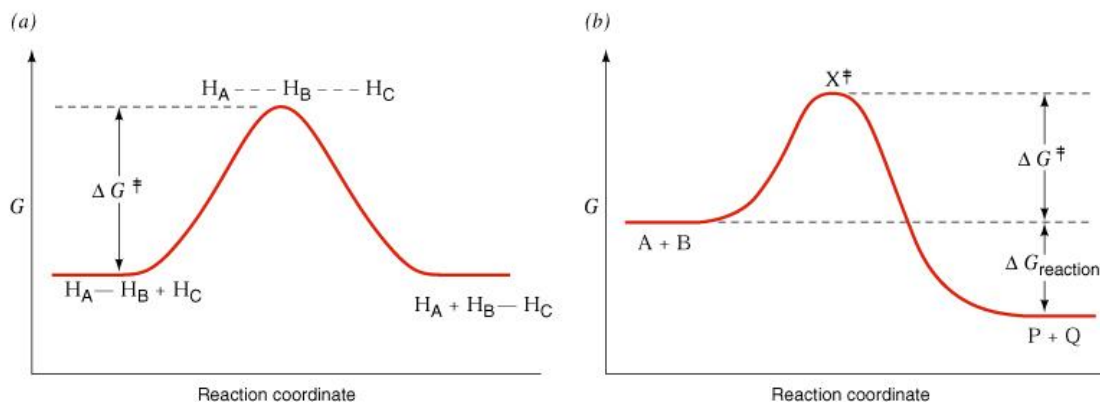
Remember this time you will be following a decrease in absorbance at 340nm due to the consumption of NADH as the reaction proceeds: You will need a blank cuvette to "zero" the spectrophotometer with since the actual reaction cuvette will have a starting absorbance; ***What do you expect the starting absorbance to be?***

What enzyme concentration range are you going to use? Malate Dehydrogenase is a very active enzyme and you should be able to make satisfactory measurements of the initial rate at concentrations as low as 0.01 μ g/mL final concentration in a 3mL cuvette. The rates will deviate significantly from initial rates if you go much higher than about 0.2 μ g/mL

The Effects of Temperature on Reaction Rate Measurements: Activation Energies

Introduction to Activation Energies

Chemical reactions proceed through transition states, where the reactant changes shape to start to resemble the product and the passage through the transition state is usually represented in an energy diagram such as:



temperature will be governed by the activation energy: the higher the activation energy, the lower the concentration of the transition state. The concentration of the transition state can thus be related to the activation energy in much the same way that the concentration of a reactant in an equilibrium mixture can be related to the equilibrium constant. Thus:

$$[A^\ddagger] = [A]_0 e^{-\Delta G^\ddagger/RT}$$

Since the rate, k , of the reaction is proportional to the concentration of the transition state, we can write:

$$k = Q_0 e^{-\Delta G^\ddagger/RT}$$

Where Q is the frequency of transition to product from the transition state, (which can of course also return to the reactant state) and is represented in units of s^{-1} . It is often more convenient to work in terms of the standard enthalpy and entropy of activation, and substituting into the above equation gives:

$$k = Q e^{-\Delta S^\ddagger/RT} \cdot e^{-\Delta H^\ddagger/RT} = Q' e^{-\Delta H_0^\ddagger/RT}$$

where Q' is a constant $= Q e^{-\Delta S^\ddagger/RT}$ and is called the pre-exponential term. This equation is the Arrhenius Equation and allows the experimental determination of ΔH_0^\ddagger from the equation:

$$\ln k = \ln Q' - \Delta H_0^\ddagger/RT$$

which predicts that a graph of $\ln k$ versus $1/T$ should be linear with a slope of $-\Delta H_0^\ddagger/RT$.

In an enzyme-catalyzed reaction the effective transition state is the enzyme bound transition state [which must have a lower energy than the free solution transition state otherwise the enzyme would not enhance the rate of the reaction!] Thus a study of the temperature dependence of the rate of the enzyme-catalyzed reaction will allow determination of the enthalpy of activation of the enzyme-bound transition state.

Determination of the Energy of Activation of the Malate Dehydrogenase Catalyzed Reaction

Using an enzyme concentration where you are sure that you are making initial rate measurements you should determine the initial rate of the malate dehydrogenase catalyzed reaction at a series of temperatures. Around the laboratory you will find either water baths or heating blocks at the following temperatures: 15°C, 30°C, 35°C, 40°C, 45°C and 50°C. Incubate three tubes of reaction mixture at each of these temperatures together with a set of three tubes at room temperature: determine the exact value of room temperature and the temperatures of the various incubations using the provided thermometers. Pre-incubate the assay mix tubes [12x75mm tubes work well] for at least 30 minutes to ensure that they have reached the desired temperatures. Removing each tube separately from the incubation area initiate the reaction with the chosen enzyme

amount and measure the initial rate of the reaction. Repeat until you have finished the complete set. Enter your results into Data Worksheet and calculate the enthalpy of activation of the reaction.

Data Worksheet 1.3

Temperature	Cuvette 1	Cuvette 2	Cuvette 3	Average	St Deviation

If you had used the oxidative reaction instead of the reductive reaction would you have expected to find the same enthalpy of activation?

Quantitative Analysis of the Data

In an experiment using an oligomeric [dimer, subunit molecular weight of 35,000] dehydrogenase, the following data was obtained when 10 μ L of an 0.01mg/mL solution was added to a 3mL cuvette containing NAD⁺ and substrate at sufficient concentrations to ensure that a maximum rate was being measured. Formation of product is measured as NADH by absorbance measurements at 340nm. [NADH has a millimolar extinction coefficient of 6.22cm⁻¹ at 340nm.]

Experiment	Temperature, °C	Measured Initial Rate, A340nm/min
1	15	0.233
2	20	0.361
3	25	0.462
4	30	0.714
5	35	0.866

Calculate the Activation Energy of the Reaction.

Using the equation:

$$\ln k_{\text{initial}} = \ln Q' - \Delta H^{\#}/RT$$

it is apparent that the activation energy for the reaction, $\Delta H^{\#}$ is obtained from a plot of

$\ln k_{\text{initial}}$ vs $1/T$ [°K]

Thus the temperature must be converted to °K [add 273 to °C] and the measured initial rate converted to a rate constant. Since rate constants for the conversion of the transition state of an enzyme catalyzed reaction to the product usually have the units time^{-1} this means calculating the initial rate in terms of M/minute and dividing by the enzyme concentration in M.

The initial rate measurements are in A340nm/min: to convert these to mM/min divide by the millimolar extinction coefficient of NADH.

The enzyme concentration in the cuvette that gave these rates is calculated in mM terms from the information given above. First calculate the enzyme concentration in mg/mL in the cuvette: 10µL of an 0.01mg/mL solution was added to a 3mL cuvette, thus the concentration in the cuvette is

$$(10 \times 0.01)/3010 = 0.0000332\text{mg/mL in the cuvette}$$

To convert this to mM the easiest way is to calculate what a 1mg/mL solution would be in mM and then simply multiply by the above number.

To calculate the mM concentration of a 1mg/mL solution start from basic principle:

A 1M solution would be 35,000gm/L or 35,000mg/mL

Thus a 1mg/mL solution is $1/35,000 \text{ M} = 0.00002857\text{M}$ or 0.02857mM

Since the enzyme concentration in the cuvette is 0.0000332mg/mL this gives:

$$0.0000332 \times 0.02875 \text{ mM} = 9.5 \times 10^{-7}\text{mM}.$$

In the table below the rates, in mM/min are divided by this concentration to give rate constants in units of min^{-1} .

Temperature, °K	Rate, A/min	Rate, mM/min	Rate Constant, min^{-1}
288	0.233	0.037	38,947
293	0.361	0.058	61,053
298	0.462	0.074	77,895
303	0.714	0.115	121,053
308	0.866	0.139	146,315

Finally construct a table containing Ln k and 1/T and plot Ln k vs 1/T

Temperature	1/T	Ln k
288	0.003472	10.570
293	0.003413	11.020
298	0.003356	11.263

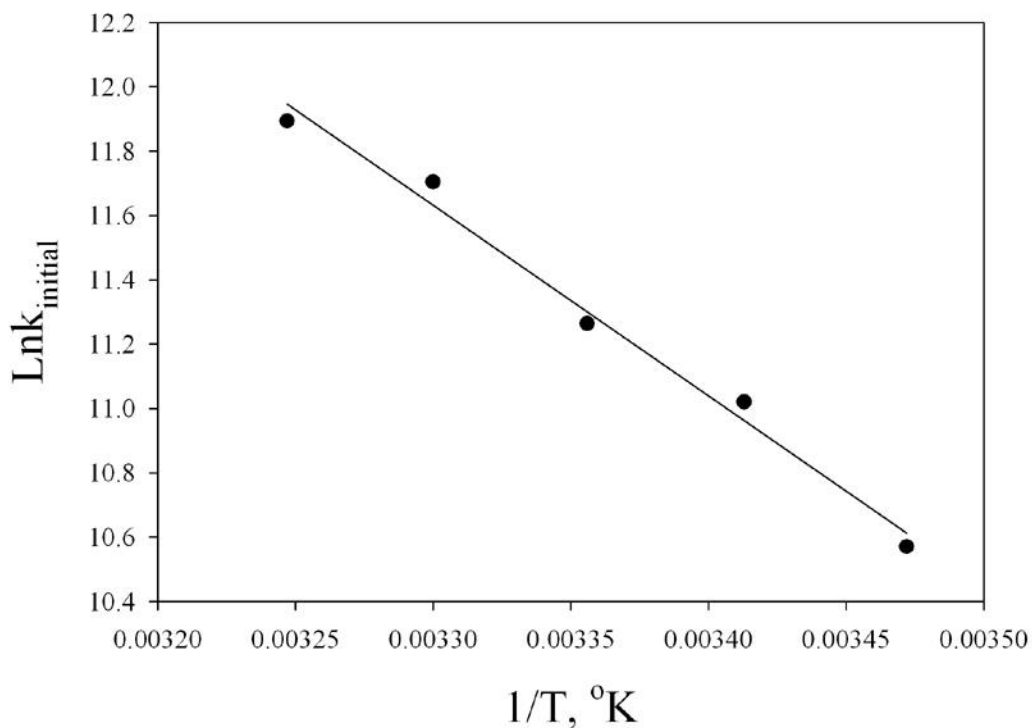
303	0.003300	11.704
308	0.003247	11.894

From the graph below, a slope of -5,927 +/- 389 and an intercept of 31.19 +/- 1.31 is obtained.

The slope = $-\Delta H^{\circ\#}/R$ and hence using a value of 8.3J/mole for R we get a value of $5,927 \times 8.3 = 49.194\text{J/mole}$ for $\Delta H^{\circ\#}$

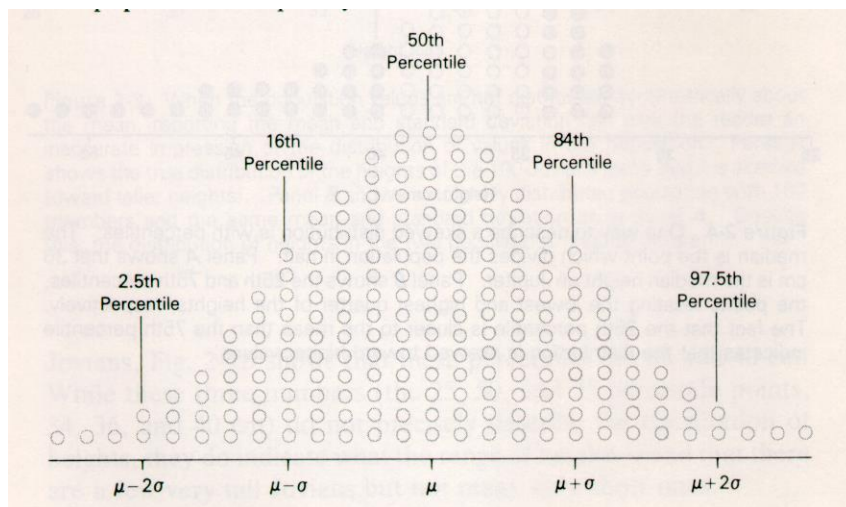
If we use a value for R of 1.92Cals/mole we get a value of 11.4 Kcals/mole for the activation energy in kcal terms.

Arrhenius Plot



Using Standard Deviations

Above we discussed putting the 95% confidence limits on a graph: where do these come from? The 95% confidence interval is the range of values that will describe 95% of all estimates of the measurement and is related to the standard deviation. Simply put 68% of the values will fall between the mean-one standard deviation and the mean+one standard deviation, while 95% of the values will fall between the mean-two standard deviations and the mean+two standard deviations. Thus using standard deviations not only tells you about how "good" the data is: a small standard deviation is "better" than a large standard deviation, but also allows the comparison of two numbers. This concept is illustrated graphically below for a "normal" distribution. Shown superimposed on this normal distribution are the so called percentile positions: the position where for example the bottom 2.5% of all estimates will fall.



The mean value is the 50% percentile and shown are the positions of the 16% and the 84%, which define \pm one standard deviation and the 2.5% and 97.5%, which define the positions of \pm two standard deviations.

If you compare two normal distributions and take 95% confidence limits: ie the values laying between the 2.5% and 97.5% values, of each distribution and the values do not overlap, there is only 2.5% of the possible estimates of the higher number that could, in a normal distribution fall into the upper echelons of the normal distribution of the lower number: the numbers would be said to differ at the level of the 95% confidence limits, or at a p value of 0.025.

This approach can be used when comparing two sets of numbers, ie means and their standard deviations or two sets of parameters from the fit to an equation, for example the slope of a line and its standard deviation.

Graphs or table of numbers should include some discussion and indication of the statistics of the data shown.

Protocol:

Using a plastic weigh boat, pipet six aliquots of the chosen volume, taring the balance to zero before each addition. Record the weights of each addition The average weight of water pipetted for each of you chosen volumes can be calculated from the formula:

$$x = \Sigma xi/n$$

where x is the mean, or arithmetic average, Σxi is the sum of the individual values, and n is the number of data values.

An estimate of the sample standard deviation, s of the values is given by:

$$s = \text{sqr root of } \Sigma (xi-x_{av})^2 / (n-1)$$

where n-1 is the number of degrees of freedom.

For example: consider that you obtained the following data for a micropipette set at 100 μ L:

Reading #	Weight, gm	Indicating Volume of:
1	0.102	0.102mL
2	0.099	0.099
3	0.103	0.103
4	0.098	0.098
5	0.101	0.101
6	0.101	0.101

The average, x_{av} , would be: 0.10067gm, indicating an average measurement of 100.67 μ L

To calculate the standard deviation:

Number	$xi-x_{av}$	$(xi-x_{av})^2$
1	1.33 μ L	1.769
2	1.67	2.789
3	2.33	5.429

4	2.67	7.129
5	0.33	0.109
6	0.33	0.109

Thus $\Sigma(x_i - x_{av})^2 = 17.334$ and $\Sigma(x_i - x_{av})^2 / (n-1) = 3.467$

Thus the sample standard deviation, which is the square root of $\Sigma(x_i - x_{av})^2 / (n-1)$ is equal to 1.86

To obtain the scatter of a small data set, the estimate of the standard deviation is multiplied by the "Student's t" factor to give the confidence interval (CI) for a single value.

You should look up a table of student's t factors and determine the 95% confidence limits for your data.

Using the Spectrophotometer

This laboratory utilizes Jasco UV-Vis Spectrophotometers. These are dual beam spectrophotometers meaning that you must use matched cuvettes, one in a reference beam and one in a sample beam: usually the reference beam will contain the appropriate buffer or blank and the sample beam, of course the sample that you are making a measurement on. There are three types of measurements that you will use almost every week of the course: fixed wavelength measurements, spectral measurements and kinetic assays or time based measurements.

Familiarizing yourself with the usage of the spectrophotometer will make your life easier. What follows are some simple guidelines to assist you.

Make sure that both the computer and the spectrophotometer are switched on! The spectrophotometer switch is on the right hand side of the box.

With both switched on you should activate the software that controls all of the operations of the spectrophotometer. Double click the icon shown below on the desktop of the computer screen:



This will bring up the operating system of the spectrophotometer [it will take several minutes to initialize the first time.

From the menu that appears select the type of measurement that you wish to make.

For Fixed Wavelength measurements you will need to set the wavelength and after you have done this you should put identical cuvettes into both the reference and the sample position and "zero" the instrument.

Fixed wavelength measurements usually are of one of two types: a) you introduce the sample to a cuvette that already contains buffer [and has been zeroed on the buffer] or you zero the instrument on buffer in the sample cuvette and then replace the solution with the solution whose absorbance is to be measured.

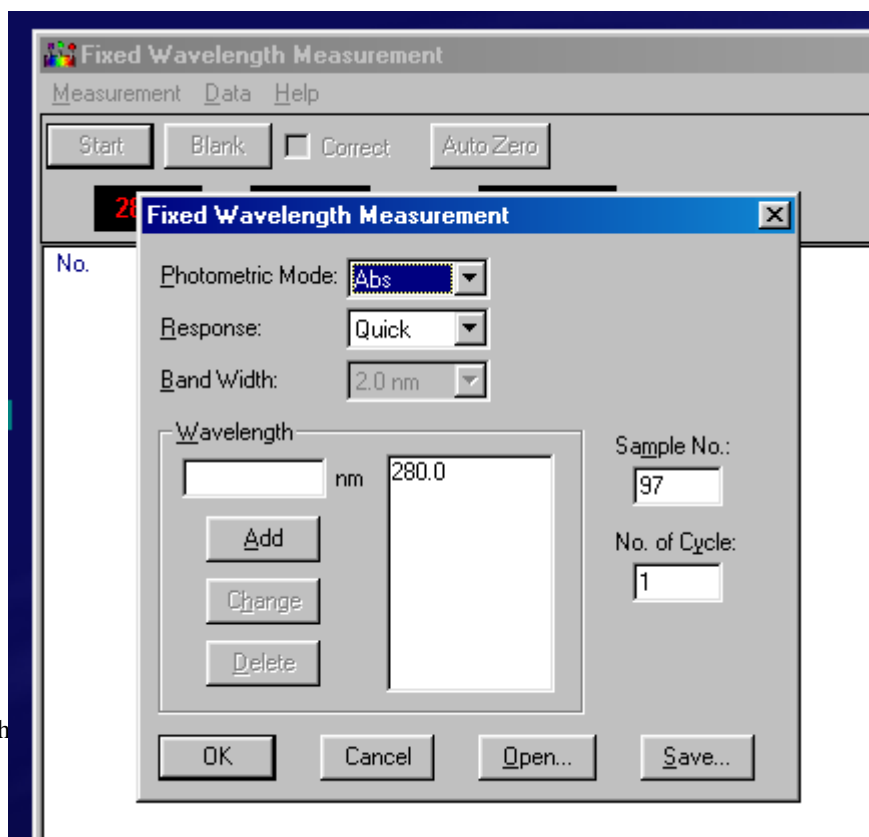
If you are using the former approach be sure to thoroughly mix the contents of the cuvette by gently "squishing" the solution up and down once or twice with a pasteur pipet.

Do not assume that diffusion, or pipetting up and down with the micropipet will be sufficient mixing: it will not!

The opening screen of the Fixed Wavelength Measurement protocol has a parameter drop down menu accessible from the "measurement" button on the menu bar:

This allows you to set the parameters that you will use. Set Abs in the top box, Quick in the next box, enter a wavelength that you want to use [or set the wavelength separately] and set the sample number at 1 [or whatever number that you wish to use].

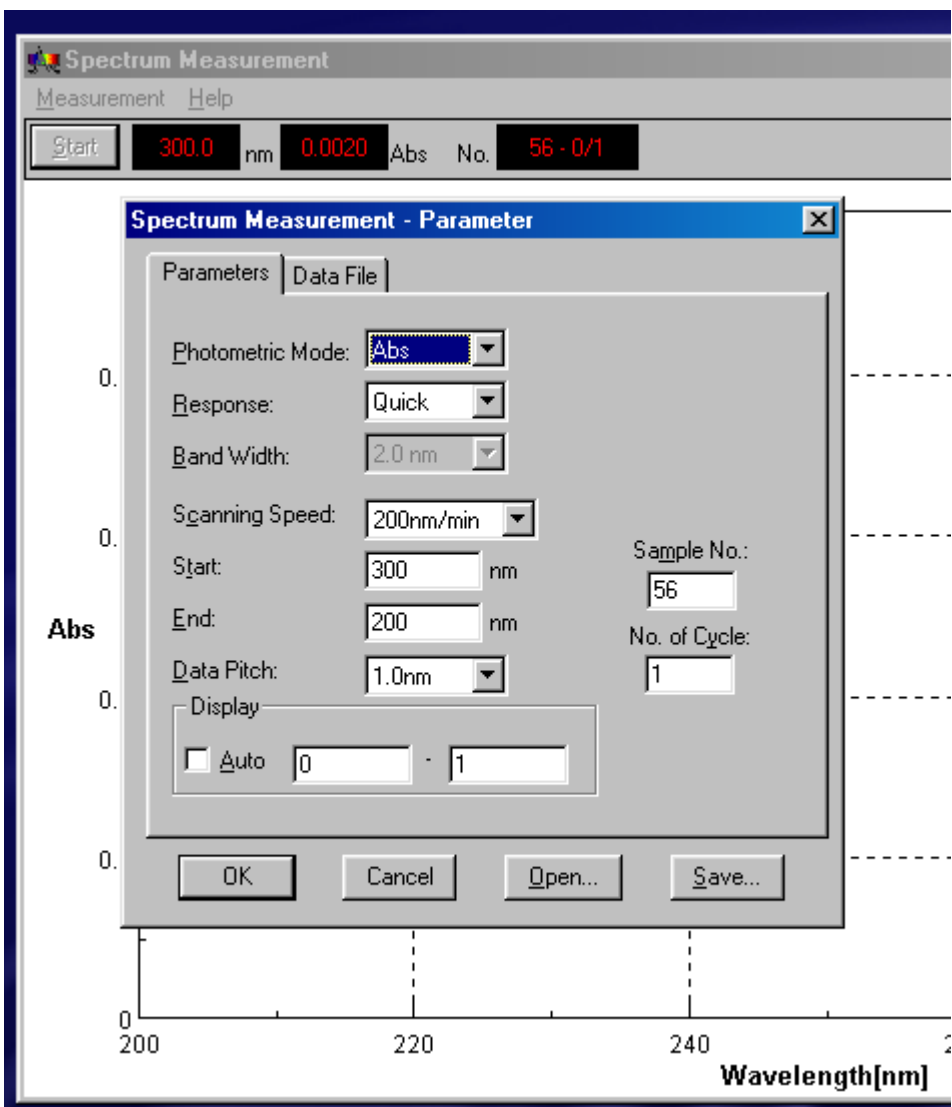
Click OK once the parameters are the way you want them. Make sure that the wavelength indicator goes to the appropriate wavelength



Making individual measurements simply involves clicking "start": the sample number and absorbance appear on the screen and can be written down in your lab note book.

Making Spectrum Measurements:

Select "Spectrum Measurements" from the opening screen and as above access the parameter drop down menu:



In addition to selecting the absorbance mode, this time you must enter starting and ending wavelengths: note that this spectrophotometer using a long start wavelength and a short end wavelength: so if you wish to scan the spectrum from 250 to 400nm, the start wavelength is 400nm and the end wavelength is 250nm. You should also select a scanning speed: a medium scanning speed works well for most circumstances: use a slow

scan speed for really accurate spectra and be aware that at the fastest scanning speeds you may get some slewing of the spectrum.

Finally select the "Data Pitch": this determines the number of data points taken. If you select 1nm it will take a reading every 1 nm through the spectrum etc.

Making Time Course Measurements:

As before access the parameter screen for time course measurements: Again set the absorbance mode etc and make sure that the wavelength is correct. Here you have two other important choices: the end time: how long do you want to follow the time course for: usually 30 seconds to a minute are sufficient for most of the enzyme kinetics in this course, and again, the data pitch: this should be set at 0.1seconds to give the maximum number of data points.

