Experiment CM-2: Mitochondrial Metabolism

Exercise 1: Calibrate the Spectrophotometer

Aim: To calibrate the spectrophotometer.

Procedure

1. With no cuvette in the holder, use the zero adjust to set the transmittance to zero.
2. Add 1.0ml of the liver extract to Tube 1 and pour the contents into a clean cuvette—this is the blank, since it contains no dye.
3. Insert the cuvette into the holder and align the marks on the cuvette and the holder. Adjust the light control to set the transmittance to 100.

Note: *You will use this "blank", Tube 1, at the beginning of each set of future measurements—do not discard!*

Exercise 2: The Reaction without Cyanide

Aim: To measure the rate of the reaction, without cyanide.

Procedure

1. Add 1.0ml of the 2,6-dichlorophenolindophenol (the dye) to Tube 2.
2. Add 1.0ml of the liver extract to tube two, place a piece of parafilm over the mouth of the tube and shake a few times.
3. Quickly pour the contents into a clean cuvette and place it into the spectrophotometer and read (and write down) the absorbance immediately and every 30 seconds for 10 minutes.

Exercise 3: The Effect of Cyanide

Aim: To measure the rate of reaction in the presence of cyanide.

Procedure

Repeat Exercise 2 using Tube 3.

Exercise 4: The Effect of a Competitive Inhibitor

Aim: To measure the rate of reaction in the presence of malonate.

Procedure

Repeat Exercise 2 using Tube 4.

Data Analysis

1. Graph absorbance as a function of time for the data from Tubes 2, 3, and 4. Use linear regression analysis to find the best line for each reaction.
2. Make a histogram to compare the rate of color change of each tube to others.

Questions

1. Look at the histogram and compare the reaction rates of Tubes 2 and 3. Comment on the function of potassium cyanide in this experiment.
2. Look at the histogram and compare Tubes 3 and 4. Comment on the effectiveness of malonate as a competitive inhibitor.
3. Is the correlation coefficient for the line graph of Tube 4 as high as the values for Tubes 2 and 3? Look at the curve for Tube 4; explain the profile in terms of competitive inhibition.

Appendix

All solutions should be refrigerated or kept on ice.

Table CM-2-L1: Recipe for Homogenizing Medium.

Concentration (mMolar)	Reagent	Grams/Liter in DI H_2O
250	Sucrose	85.57
0.01	EDTA	0.00292
15.0	Tris-HCl	2.36
200	Sodium Succinate	54.0
200	Sodium Malonate	29.6
Adjust the pH of the Medium to 7.4		

Table CM-2-L2: Recipe for SPT Buffer.

Concentration (mMolar)	Reagent	Grams/Liter in DI H_2O
250	Sucrose	85.57
20.0	K_2HPO_4 Anhydrous	3.48
15.0	Tris-HCl	2.36
Adjust the pH of the Medium to 7.4		

Table CM-2-L3: Concentrations of Reagents that Affect Mitochondrial Respiration.

Concentration (mMolar)	Reagent	Grams/Liter in DI H_20
200	Sodium Succinate	54.0
200	Sodium Malonate	29.6
50.0	Potassium cyanide	3.256
0.10	6-Dichlorophenolindophenol	0.0268