

Succinate
dehydrogenase
enzyme in inner
mitochondrial
membrane biology
essay



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Succinate dehydrogenase (SDH) is an enzyme found in the inner mitochondrial membrane, which makes it an easy target to isolate when studying the citric acid cycle. This enzyme is responsible for catalyzing the oxidation of succinate into fumarate and can be used as a marker enzyme during the isolation of mitochondria through differential centrifugation. The isolated mitochondria can be treated with a sodium azide reagent to inhibit the mitochondrion transport of electron in the cell extract. To measure the activity of the enzyme, an artificial electron acceptor (2, 6-dichlorophenolindophenol, DCIP) is used to accept two electrons. Upon receiving electrons, the oxidized DCIP is reduced and the color of the mixture changes from blue to colorless. Spectrophotometry at the 600nm range can then be used to quantify this color change, and give an indication of the mitochondrial content of a given sample. As the The findings show that the experiment mimics Michaelis-Menten kinetic properties

Enzymes are regulators of metabolic pathways that lower the activation energy in order to catalyze the acceleration of biochemical reactions [1]. Most enzymes are characterized as showing Michaelis-Menten (M-M) kinetic properties. Simply, enzymes work by binding its substrate reversibly changing its conformation to form an enzyme-substrate complex, and then detach to form free enzyme and product. If there is low substrate concentration, there will be very little enzyme activity and the rate of the reaction will slow down. If there is high substrate concentration, the enzyme will be more active and the reaction will be faster. At a certain point, if the substrate concentration is saturated, the rate of the reaction will not increase [1]. Along with the substrate concentration, these dynamics can be

characterized as the M-M constant (K_m) and maximum velocity (V_{max}). These factors determine the initial velocity of the biochemical reaction and contribute to the understanding of the M-M equation (in fig. 1) However, when a competitive inhibitor is present, the inhibitor can bind to the active site to prevent the normal substrate from binding and forming the product. Thus, both the inhibitor and substrate compete for the active site of the enzyme, which based on the M-M equation, allows the V_{max} to stay constant and the K_m to change [2].

In the experiment, we will examine activity of SDH, an important component of the citric acid cycle that is responsible for catalyzing the oxidation of succinate to fumarate in the inner membrane of the mitochondria. The enzymatic activity will be determined by mitochondria fractionation from isolated cells of cauliflower by the technique of differential centrifugation. Also, we will determine the effects of enzyme concentration and competitive inhibition on the initial velocity of the reaction by adding the malonate, a classic competitive inhibitor. We will measure the reaction by blocking the electron transport with sodium azide and monitoring the reduction of the DCIP that can be followed by the change in spectrophotometry absorbance reading at 600 nm over time. Since the oxidized form of the dye is blue and the reduced form is colorless, the reaction can be reestablished based on the experiment (in fig. 2). Thus, we hypothesize that the reaction will follow M-M kinetics as the absorbance will decrease when the malonate is added.

Methods

In isolating mitochondria, we removed with a scalpel 20 g of cauliflower from the outer 2-3 mm surface. Then, we grinded the tissue with a pestle in a chilled mortar in 40 ml of ice-cold mannitol grinding buffer for 4 min. We filtered the suspension and squeeze the solution out through four layers of cheesecloth into three chilled 15 ml centrifuge tube. Then, we centrifuged the filtrate solution at 1000 x gravity for 10 min and decanted the supernatant into a chilled 50 ml centrifuge tube. After, we re-spun the filtrate solution at 10, 000 x gravity for 30 min at 0-4°C and discarded the supernatant in the sink leaving the pellet. Then, we added 7. 0 ml of icecold mannitol assay buffer to the mitochondrial pellet and scraped and mixed the mitochondrial pellet from the wall of the centrifuge tube with a spatula and vortex thoroughly to re-suspend the pellet in the assay buffer. Until needed, we transferred the mitochondrial suspension to a test tube and stored it in an ice bath.

In measuring the activity of SDH, we label 10 test tubes or cuvettes as shown in table 1. We heated 0. 6 ml of the ice cold mitochondria suspension in a boiling water for 5 min and placed it in an ice bath to cool. Then, we added correct volumes of azide, DCIP, malonate, and succinate to all labeled test tubes indicated in the table, covered them with Parafilm and inverted to blend the solutions. After, we add specific volume of the mitochondrial suspension to blanks 1-4 and tubes 1-4. Using a spectrophotometer set at 600nm, we blanked and took the absorbance of tubes 1-4 every two minutes until 20 minutes after the first reading. Then, we repeated again by taking the absorbance using only test tubes 5-7 for every two minutes.

Results

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The spectrophotometer results we obtained are presented in Table 2, and shown graphically in Figure 3-7. In Table 2, the first 4 test tubes and blanks we were only able to take 3 readings and the test tubes 5-7, we were able to take 4 readings. The greatest absorbance reading was obtained for test tube 4 at 2.363 abs., which is because malonate, the competitor inhibitor, is present along with the substrate, succinate. In Tube 6, one of the lowest absorbance readings because it is a negative control and does not have any cellular suspension. This is shown experimentally when the reaction mixture will remain the color blue because with the succinate there is no reaction between the marker enzyme and the DCIP. In Table 3, we calculated the change in absorbance from tube 1-4 for every 2 minutes. We also calculated the initial velocity by dividing the change in absorbance by the elapsed time. In Figure 3, the graph shows the initial velocity depends on the enzyme concentration. When the enzyme concentration is high, it starts to rapidly decrease the initial velocity because the ratio of substrate to enzyme will be abnormally low, which will decrease the formation of product. In Figure 4, the graph represents the data in Table 2, where the initial velocity is measured by elapsed time. The second highest reading was found for Tube 2 (0.987), which was also in concordance with the class results. This sample contained the heaviest constituents of the cell (mostly nuclei), as well as any unbroken whole cells that may have remained after the mechanical grinding and initial centrifugation at 600x. We found Tube 8 to have the third highest absorbance reading (0.626) and Tube 4 with the lowest (0.483). However, the sample from Tube 8 should have had a lower absorbance value than Tube 4, as was seen in the average class results displayed in Table 1. Tube 8 should contain the majority of the mitochondria (as well as some lysosomes), <https://assignbuster.com/succinate-dehydrogenase-enzyme-in-inner-mitochondrial-membrane-biology-essay/>

and Tube 4 should have any residual mitochondria and smaller organelles that did not remain in the pellet after the 12, 000x centrifugation.

Discussion

There are a number of reasons why our findings did not match up with the expected results. Although improbable, it is possible that the 12, 000x centrifugation for 30 minutes was not properly carried out, perhaps because the samples were not maintained at a consistent temperature of 0-40C. It is more likely that the re-suspension of the pellet (Tube C) with the mannitol assay buffer was not performed effectively. The pellet clumps may not have been properly dispersed, and so even though more mitochondria may have been present in Tube 8 (as they should have been), they were not free to interact with the other reagents in solution. A third reason may be that too much DCIP was added to Tube 8 (relative to Tube 4), and so there was an excess of the blue DCIP reagent in that sample (and hence a higher absorbance reading due to a lower degree of color loss). Differential centrifugation, when done correctly, is a reasonably effective method for mitochondrial isolation, although separation is achieved based only on size differences of the cell components. When dealing with small organelles, a more appropriate method to use may be sucrose gradient centrifugation, which allows for separation based on size as well as shape, especially when dealing with crude cellular extracts such as cauliflower.

Cited Resources

Nelson, D. L., Cox, M. M. (2007) Lehninger: Principles of Biochemistry, Fifth Edition, Freeman, New York, NY
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Gilbert, H. F. (2000) Basic Concepts in Biochemistry, Second Edition, McGraw Hill, New York, NY

Figure 1

Figure 2

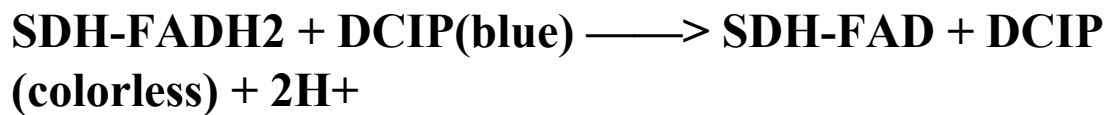


Table 1

Cuvette

Assay Medium

Azide

DCIP

Malonate

Succinate

Mitochondrial Suspension

Blank 1

3. 7 mL

0. 5 mL

—

—

0. 5 mL

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0.3 mL

1

3.2 mL

0.5 mL

0.5 mL

—→

0.5 mL

0.3 mL

Blank 2

3.1 mL

0.5 mL

—→

—→

0.5 mL

0.9 mL

2

2.6 mL

0.5 mL

0.5 mL

—

0.5 mL

0.9 mL

Blank 3

3.4 mL

0.5 mL

—

—

0.5 mL

0.6 mL

3

2.9 mL

0.5 mL

0.5 mL

—

0.5 mL

0.6 mL

4

2.7 mL

0.5 mL

0.5 mL

0.2 mL

0.5 mL

0.6 mL

5

3.4 mL

→

0.5 mL

—

0.5 mL

0.6 mL

6

3.4 mL

0.5 mL

0.5 mL

—

—

0.6 mL

7

2.9 mL

0.5 mL

0.5 mL

—

0.5 mL

0.6 mL

Table 2

Trial 1

Trial 2

Trial 3

Trial 4

Blank 1

0.04

0.003

0.006

0.008

1

1.10

1.16

1.158

1.112

Blank 2

0.03

0.001

0.004

0.003

2

0.64

0.644

0.648

0.645

Blank 3

0.06

0.002

0.008

0.005

3

0.56

0.06

0.670

0.682

4

2.36

1.85

2.221

2.223

5

0.83

0.73

0.723

0.720

6

0.76

0.73

0.734

0.725

7

0.78

0.72

0.704

0.705

Table 3

Time (min: sec)

Tubes 1-4

\hat{A} †Abs (nm)

Initial Velocity

(Δ Abs/min)

Time (min: sec)

Tubes 5-7

Δ Abs (nm)

Initial Velocity (Δ Abs/min)

11: 10

-. 5505

-. 0500

9: 10

. 1057

. 0117

13: 10

-. 0008

-. 00062

11: 10

. 032

. 0029

15: 10

. 4989

. 03326

13: 10

. 0617

. 0047

17: 10

. 5062

. 0298

15: 10

. 1161

. 00774

19: 10

-. 0533

-. 0028

17: 10

. 0338

. 00199

21: 20

-. 0043

-. 0002

19: 10

. 0784

. 0041

23: 10

-. 1095

-. 0048

21: 10

. 1195

. 0057

25: 10

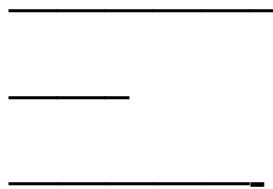
. 142

. 00568

23: 10

. 0428

. 0019



25: 10

. 0781

. 0031

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7