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Structure and function of aconitase enzyme in TCA by estimating optical rotation of glucose and determining the relationship between cell density and absorbance

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Abstract

Enzyme aconitase have a great value in TCA path, this enzyme use to convert pyruvate and acetyl co A in to citrate and cis aconitase (a six carbon molecule). This study was designed to find the tertiary structure of aconitase with and without ligand by using RSBC. The TCA cycle start with the pyruvate that is end product of glycolysis cycle. This study also focused on the optical rotation of glucose molecule before its breakdown start naturally through glycolysis and absorbance / transmittance of viable cells would be estimated**.**

Keywords: Enzyme aconitase, glycolysis cycle, Citrate.

1. Introduction

Enzymes in our body work as a catalyst. They perform major role in metabolism. One enzyme can perform two different tasks. Such enzymes having same amino acid sequence but performing different functions e.g. aconitase enzyme present in mitochondria participate in kreb cycle, it converts citrate in to aconitaste, an intermediate and finally converted in to isocitrate [1]. Iron regulatory protein 1 is use to control the level of iron in the cells, this form is present in cytosol and can be converted into other form [2]. Another example of this is phosphorgluco isomerse enzyme takes part in glycolysis, performing another job as well its act as a cellular messenger. Cytocrome c is an ETC carrier but having anther task with same amino acid sequence that is when cell is damaged it response apoptosis. Retinal dehydrogenase enzyme present in retina its job is to convert the light sensingretinal into regulatory molecule retinoic acid with NADH cofactor. Its second job is to modify the consistency and absorbance of eye lenses where it's called eta-crystallin [3].

Cell density refers to the number of cells per unit volume. Often cell density is denoted as viable cell density which is the number of living cells per unit volume [4]. A spectrophotometer is employed to measure the amount of light that a sample absorbs**.** It used to calculate two quantities: the transmittance (T) and the absorbance (A) [5].

The photons in plane polarized light only oscillate in one direction. If this plane polarized light is passed through a solution of optically active molecules, the degree and direction of rotation changes [6]. The specific rotation for a given wavelength λ, at a given temperature and solvent is defined by the relation [7].

$$[\alpha]_{\lambda}^{t} = \frac{100\alpha}{l\,c} =$$

Observed rotation/length (dm) × concentration (gm/ml)

α is the observed angle of rotation (degrees),
t is temperature (^{o}C),
λ is the wavelength of light used (nm)
l is the length of polarimeter tube (dm) = 100 mm = 1 dm
c is concentration in g solute/100 mL solution

2. Material and Methods

First of all, two solutions of glucose were prepared A & B of different concentration. The prepared solutions

were 0.01 M (A) and 0.001 M (B). Then, I filled the tube with glucose sample solution of 0.01 M. I assured that there was no air bubble in the tube. And put the sample inside the chamber. Put on the sodium lamp and recorded the readings.

The culture was diluted in water using dilutions of 20%, 40%, 60%, and 80%. Measurements should be made of the undiluted and diluted samples at 440nm and 660nm using the spectrophotometer. There should be no sample in blank it only contains water. We should make volume up to 1 ml.

Dilutions: 0.2ul of water + 0.8ul of stock = 1ml
0.4ul of water + 0.6ul of stock = 1ml
0.6ul of water + 0.4ul of stock = 1ml
0.8ul of water + 0.2ul of stock = 1ml

The structural studies of aconitase enzyme were done by using RCSB tool. Ball and stick structure was extracted, Pleated sheets and helix were studied with addition to absence of ligand molecule.

3. Results and Discussion

Aconitase enzyme used citrate as a substrate in citric acid cycle and converts it in to isocitrate**.**

Citrate ---------- aconitate ---------- isocitrate

The same amino acid **se**quence enzyme called iron regulatory protein 1 is present in cell cytosol and control iron level. The cytosolic form has one protein chain that fold into domains [8]. These domains folds around the active site of iron sulfur cluster. When the amount of iron-sulfur is low in the cell, this enzyme converts it into another form because there is no iron present to regenerate clusters and then proceed with different substrate. Regulatory protein 1 attaches with hairpin loop of mRNA to enhance the formation of transferin receptor (it picks out transferin out of blood and with it more iron) it inhibits the formation of ferratin so that less iron is locked in storage [8]. Aconitase perform stereospecific reaction it take OH- group and hydrogen from citrate and replaces to form aconitate later OH and H is added back to form isocitrated this is done by aconitase in mitochindria but in cyrosol it extract oxygen and H by a serine amino acid. The serine is mutated to alanine.

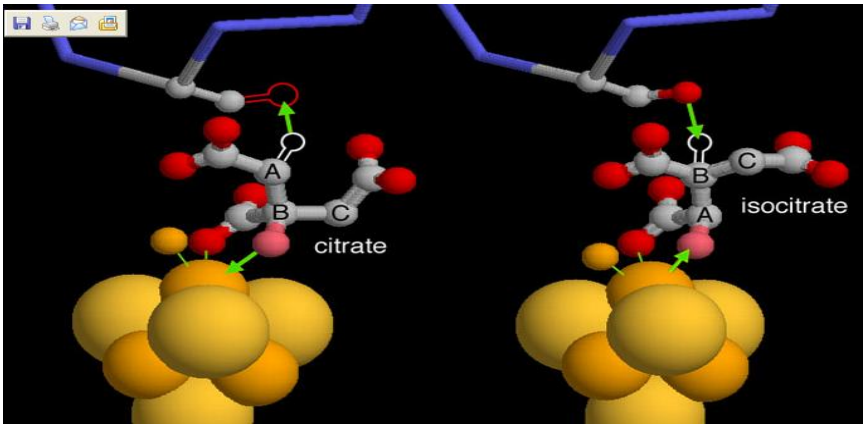


Figure 1: Cartoon (secondary structure having peptide bond van der waals forces) it has beta pleated sheets and alpha helix

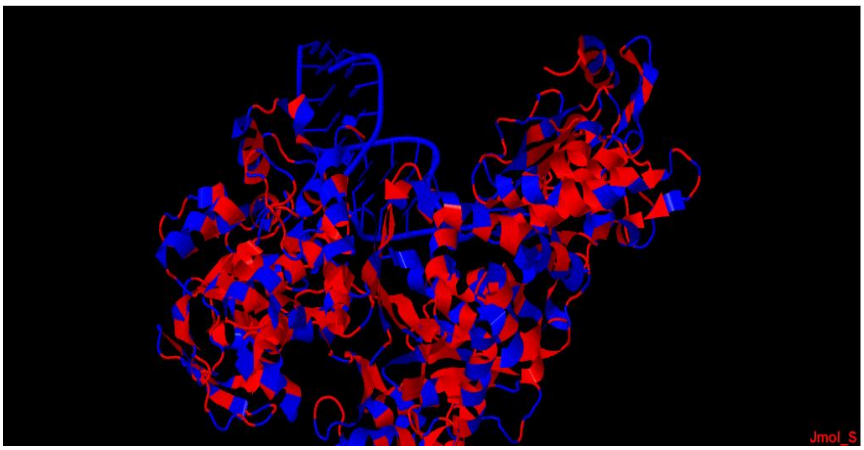


Figure 2: Blue in color is the hydrophilic amino acids (direct contact with water) and red color shows hydrophobic amino acids which do not have direct contact with water.

For glucose sample solution A (0.01 M)

α = 0.0135˚
λ =589 nm
l = 100 mm
t = 25.8˚ C
c = 0.01M = 1 gm/ 100 ml

As the formula for finding the optical rotation is

$$[\alpha]^t_\lambda = \frac{100\alpha}{l\,c} =$$

Observed rotation/ length (dm) × concentration (gm/ml)

Putting the values, we get

$$[\alpha]^t_\lambda = \frac{100\alpha}{l\,c} = 0.0135^\circ / 100\text{ mm} \times 1\text{gm}/ 100\text{ ml}$$

α =0.000135˚

For glucose sample solution B (0.001 M)

α = 0.183˚
λ =589 nm
l = 100 mm
t = 25.8˚ C
c = 0.001M = 10 gm/ 100 ml

Putting the values, we get

$$[\alpha]^t_\lambda = \frac{100\alpha}{l\,c} = 0.183^\circ / 100\text{ mm} \times 10\text{ gm}/ 100\text{ ml}$$

= 0.000183˚

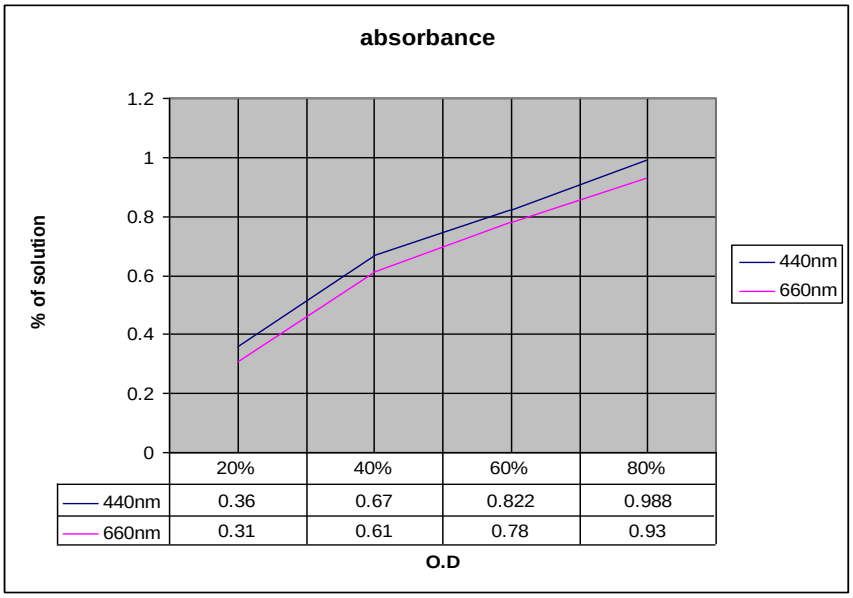
Optical rotation of Glucose of 0.01 M = 0.000135˚
Optical rotation of Glucose of 0.001 M = 0.000183˚

Results

Table 1: Absorbance at 440nm and 660nm

% solution (X-axis)	O.D 440nm(y-axis)	O.D 660nm(y-axis)
20%	0.360	0.310
40%	0.670	0.610
60%	0.822	0.780
80%	0.988	0.930

Figure 3: Absorbance at 440nm and 660nm



The measured absorbance in these turbid samples is due to light scattering, and not the result of molecular absorption. The instrument used to measure turbidity is a spectrophotometer [10]. It consists of a light source, a filter which allows only a single wavelength of light to pass through the sample tube containing the bacterial suspension and a photocell that compares the amount of light coming through the tube with the total light entering the tube. The ability of the culture to block the light can be expressed as either percentage of light transmitted through the tube or the amount of light absorbed in the tube [11]. The percent of light transmitted is inversely proportional to the bacterial concentration. Greater the percentage of transmittance, the lower the number of bacteria. The absorbance or optical density is directly proportional to the cell concentration [12].

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