TARDIGARD

protocol

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CAHS can increase desiccation tolerance in vitro with a Spectrophotometric Stop Assay

1. Objective

To prove that Invertase activity can be preserved as function of increasing CAHS concentration

1. Description

The official name for invertase is (b-fructofuranosidase, EC 3.2.1.26 http://enzyme.expasy.org/EC/3.2.1.26) It catalyze the hydrolysis of sucrose to fructose and glucose by cleaving the O-C bond on fructose. The resulting sugar are reducing sugar, and when warming with 3,5 dinitrosalicylic acid(DNS) will produce a orange precipitate which can be detected colorimetrically. The DNS does not react with sucrose, so it will not contribute to the absorbance measured by the spectrophotometer. We can thus measure the activity of invertase by observing the formation of catalyzed reaction product

1. Invertase Unit Definition

One unit of invertase catalyse the formation of 1 µmole glucose in a minute at pH 4.5, temperature 25C (international units (IU) of enzyme were defined as the amount of product (in µmole) released per minute under experimental conditions)

1. Procedure

a. Materials needed

Invertase

BSA

Purified CAHS

a. Reagents need to be prepared

i. pH 4.5 buffer

ii. DNS reagent

One gram of dinitrosalicylic acid, 200 mg of crystalline phenol, and 50 mg of sodium sulphite were simultaneously dissolved in 100 mL of 1 per cent NaOH solution by stirring.

+ 40% Rochelle salt solution (sodium-potassium tartrate solution)

i. 5% sucrose solution

ii. 1g/ml standard glucose solution

a. Method of standard curve production

i. Standard curve was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the standard glucose solution and the volume was made up to 3 mL by adding distilled water

ii. 3 mL of DNS reagent was added and the mixture was heated for five minutes in a boiling water bath

iii. After the development of the color, 1 mL of 40% Rochelle salt solution was added (in warm contents) and mixed thoroughly

iv. cooling the tubes by washing it with tap water(一定要确认降到室温，否则会影响读数）

v. Recorded absorbance at a wavelength of 540 nm

a. Method of testing

i. Enzyme + 5% sucrose solution

3 groups: buffer(control) ,BSA, varying concentration of CAHS (depends on purified protein conc)

i. Made up to 3ml by adding ddH2O

ii. Water bath at 30C for x minutes(x=0.5, 1, 1.5, 2……..10)

iii. 3 mL of DNS reagent was added

iv. Heat the mixture for five minutes in a boiling water bath

v. The reaction was terminated by the addition of 1 mL of Rochelle salt solutionas soon as color appears

vi. After cooling, the absorbance was recorded at 540 nm

Notice

- Rochelle salt is added to prevent solution dissolving oxygen, stabilising the color
- However it interferes with the protective action of sulfide, which removes the dissolved oxygen from solution, so it need to be added immediately after the color appears

(1)Department of Microbiology, College of Basic Sciences, Himachal Pradesh Agricultural University ,Materials and Methods
(2)Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar G.L.Miller
(3)Zbid.,62, 287 (1924-25)