Himanshu - 19-20
Art

Modern Research in Botany

Vol. 1

India ▪ United Kingdom

Book Publisher International

Editor(s)

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FIRST EDITION 2020

ISBN 978-93-89246-10-0 (Print)
ISBN 978-93-89246-31-5 (eBook)
DOI: 10.9734/bpi/mrb/v1

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Print ISBN: 978-93-89246-10-0, eBook ISBN: 978-93-89246-31-5

Analysing Activity of Cellulase and Macerating Enzyme and Effect of Catechin on Cellulase Activity

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DOI: 10.9734/bpi/mrb/v1

ABSTRACT

Cellulose having linkages like β-1,4-linked homopolymer of glucose is major and primary portion in plant cell wall. Detection of released monosaccharides after enzyme treatment with suitable required cell ambience can unleash the detailed mechanism of optimum activity of cellulase and macerating enzyme. Efficiency of enzyme effect can be studied on sample plant particles. Cellulases and macerating enzyme hydrolyse cellulose into monosaccharides like glucose, dextrose and fructose. Cellulase activity may be affect by presence of catechin, thus catechin effect can be either inhibiting or facilitating the reaction. The detailed mechanism is discussed in the article.

Keywords: Cell wall; cellulase; cellulose; macerating enzyme; catechin; monosaccharides.

1. INTRODUCTION

Cellulose, hemicelluloses and pectin are the polysaccharides found in building blocks of plant cell wall. Storage polysaccharides like starch, inulin and gums are also found in plant. Each one of them are made of many different monomeric components attached to each other by different types of linkages [1,2]. Structural integrity to cell wall in plant is due to presence of cellulose which is the most abundant carbohydrate formed of β-1,4-linkages homopolymer of glucose and can be broken down by enzyme cellulases [3,4]. Linkages like β-1,4 glycosidic bonds links D-glucose subunits forming cellobiose molecules which are linear polymer. All these chains are linked together by hydrogen bonds and van der Waals forces [5]. Some amounts of non-organized cellulose chains are also found, and they form amorphous cellulose. Wood dry weight can be due the cellulose as its proportion is around 45%. Thus, cellulose can be degraded enzymatically as it is more functional. Linkages like β-1, 4 and occasionally β-1,3 glycosidic bonds are found between the sugar's molecules. Thus by enzymatic action, the cell wall can be broken down and inner content of plasma cytosol can be readily extracted [6,7,5]. Hence, economical target product can be extracted in simple and fastest mode for maximum extraction of it.

Enzymatic action of cellulase and macerating enzyme on the cell wall can be used to break down the linkages as together they act as hydrolysing enzymes. Macerating enzyme is a mixture of commercially available cellulase and hemicellulase. Both presences can be beneficial as the enzyme activity is divided in two steps that is degradation of cellulose first by a pre-hydrolysing where anhydroglucose chains are hydrated. Followed by the degradation of polymers by hydroxylation occurs in random or at polymer ends. They work together with including exoglucanase, endoglucanase and β-glucosidase (cellulase complex) to hydrolyze cellulose. Enzymes can be into β-1,4-endoglucanases, β-1,4-cellobiohydrolases, β-glucosidase, β-mannanase and α-glucuronidase [6]. A true cellulase can convert crystalline or amorphous cellulose to glucose very efficiently. It is well known that multi-enzymatic system is plays an essential role in the overall process of converting cellulose to glucose [8,9]. So, we hypothesize that the degradation of plant structural integrity can be helpful for obtaining maximum targeted inner metabolite of plant cell.

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Similar structure is found in the catalytic domain of cellulase and macerating enzyme molecules like cellulose-binding domain and domain bridge connecter. Thus, cellulose can be broken down to glucose with the help of these enzymes in a synergistic action. Cellulases degrade cellulose into glucose, dextrose and fructose which helps in improving inner metabolite extraction by hydrolysing the plant cell walls [6,9].

With the help of product estimation at different temperature and pH, the enzyme activity of cellulase and macerating enzyme was determined by using Miller's DNSA method (1972) and Rajbhar et al. [10]. Also, the effect of catechin dose on cellulase activity was studied in the enzymatic reaction.

2. MATERIALS AND METHODS

Optimal activity of both the enzyme in different pH and temperature was determined by estimating sugar products like glucose, dextrose and fructose. Standard substrate was cellulose for enzyme study, collected data was used for linear curve plotting. By using Rajbhar et al. [10] modified DNSA method the optimal pH and temperature was studied. Thus, the activity optimisation was calculated by the amount of sugar product released in equivalence to glucose, dextrose and fructose against a known amount of substrate cellulose.

2.1 Chemicals & Instrument Used

Dinitrosalicylic acid (DNSA) and crystalline phenol were used in the study was obtained from HI-Media (India); Cellulose, potassium sodium tartarate (Rochelle salt), sodium sulphite and sodium hydroxide from Loba Chemie (India). Cellulase and macerating enzyme used for the study was from Sigma (India) and Novozyme (India). Instrument used were water bath (Equitron), microwave (LG) and Jasco UV-VIS V-530 spectrophotometer.

2.2 DNSA Reagent, Substrate Solution and Enzyme Solution Preparation

Dinitrosalicylic Acid Reagent (DNSA Reagent) was prepared by using 1 g DNSA, 200 mg crystalline phenol and 50 mg sodium sulphite in 1% NaOH 100 mL, and shelf life was increased by storing at 4°C. The reagent deteriorates due to sodium sulphite, so it was added at the time of use to qualify prolonged storage, prior to the addition of 40% Potassium sodium tartrate (Rochelle salt solution).

Cellulose was solubilised in luke warm distilled water for preparation of mg/ml clear substrate solution. Enzyme stock solution was prepared by mg/ml solution in distilled water and later in sodium acetate buffer of respective pH buffer.

2.3 Reaction Mixture Preparation

A total volume of 2 ml solution with 0.1 ml enzyme volume suspended in corresponding buffer making a volume of 1.9 ml followed by 0.1 ml of substrate solution. Incubation of 30 mins at 40°C was given to reaction mixture at room temperature. Later, 0.5 ml DNSA reagent was added and the mixture kept in water bath at 85°C for 15 min. When the contents of the tubes were still warm, 0.5 mL of 40% Rochelle salt solution was added. This reaction mixture was cooled, and absorbance of the coloured complex formed was measured at 456 nm, 455 nm and 453 nm in terms of glucose, dextrose and fructose equivalence using a Jasco V-530 spectrophotometer [11,10,12,13]. Standard graph was plotted with monosaccharide equivalence concentration (microgram) on Y-axis against respective parameter on X-axis.

2.4 Standardisation of Optimum pH and Optimum Temperature

Preparation of substrate cellulose with cellulase and macerating enzymes for reaction mixture, at different pH of 0.1 M sodium acetate buffer and at different temperature in pH 6.0 and pH 3.8 sodium acetate buffer (0.1 M) resulted to sugars product estimation by DNSA method in glucose equivalent (456 nm), dextrose equivalent (455 nm) and fructose equivalent (453 nm) [10,12].

dextrose equivalence (DE) and fructose equivalence (FE). Statically given codes (A, a and M) shows the significance of temperature effect was at 40°C which is best suited for macerating enzyme activity. (Note: - Statistic code in ascending order).

3.1 Statistical Analysis of Data

SPSS version 22 was used for statistical analysis of the data obtained. The reported values are the of mean n (n=3) ±SD. The results of the analysis were obtained for $P<0.05$. In cases where ANOVA was performed, multiple assessments were made using Duncan's Multiple Range Test (DMRT). Glucose equivalents (GE), dextrose equivalents (DE) and fructose equivalents (FE) for reducing sugar and gallic acid equivalents (GAE), catechin equivalents (CE), quercetin equivalents (QE), rutin trihydrate equivalents (RTE) and ascorbic acid equivalents (AAE) series have been assigned to groups using upper case letters (A>B >C...) (M>N>O...) as well as lower case (a>b>c...) as per the needed requirement in graphs. Highest value reported in ascending way as A> AB> ABC> ABCD> ....> B>BC>BCD>..... same method with lower case alphabets were used for differentiation purpose. In a given series, mean assigned the same letter(s) are not significantly different from each other $P<0.05$.

4. DISCUSSION

Cellulase degrade cellulose polysaccharides into glucose, fructose and dextrose. Thus, quantification of the amount of degraded product at various pH of sodium acetate buffer (SAB) by using 10 µl cellulase on 100 mcg cellulose have released product in 0.033±0.0002 mcg of glucose, 0.031±0.0013 mcg of dextrose and 0.047±0.0008 mcg of fructose equivalence at room temperature in pH 6.0 of sodium acetate buffer 0.1 M at RT as shown in Fig. 1. Optimal cellulase activity in SAB pH 6.0 was observed at 40°C which showed the product release of 0.043±0.0006 mcg of glucose, 0.051±0.0012 mcg of dextrose and 0.054±0.0002 mcg of fructose equivalence (Fig. 3).

Macerating enzyme is a combination of cellulase and hemicellulase. It is well observed in graphs 1.1, 1.2 and 2.1, 2.2 that macerating is unlike with cellulase activity. The synergic effect is all together diverse as shown in Fig. 2 i.e. 100 µl of macerating enzyme activity on 100 mcg cellulose as the highest activity in 0.1 M sodium acetate buffer in pH 3.8 at room temperature by releasing 0.204±0.008 mcg of glucose, 0.207±0.0064 mcg of dextrose and 0.188±0.0079 mcg of fructose equivalence at RT in pH 3.8 of sodium acetate buffer 0.1 M at room temperature. Optimal macerating activity in SAB pH 3.8 was obtained at 40°C with the release of 1.279±0.0224 mcg of glucose, 1.141±0.0520 mcg of dextrose and 1.568±0.039 mcg of fructose equivalence as observed in Fig. 4.

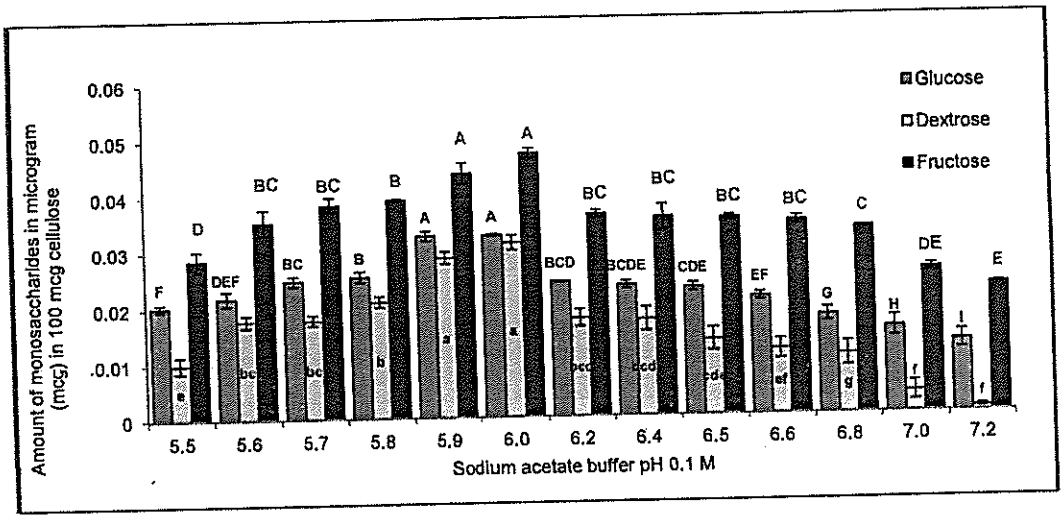

Fig. 1. Cellulase enzyme activity on cellulose in sodium acetate buffer

mixture lower the activity of an enzyme. Higher amount of catechin can facilitates the activity of the enzyme, which gradually increases the reaction between enzyme and substrate.

5. CONCLUSION

Cellulase and macerating enzymes activities on dried leaf powdered can be observed in Figs. 5, 6 & 7. Amount of product sugar in glucose, dextrose and fructose equivalence is shown in Figs. 6 & 7 before treatment i.e. control (c-GE, c-DE & c-FE). When plant material was treated with enzymes there was consistent increase in glucose, dextrose and fructose equivalence. Thus, enzymes work best on leaf polysaccharide at 40°C in sodium acetate buffer, in pH 6 for cellulase and in pH 3.8 for macerating enzyme. Release of polyphenols and flavonoids with microwave-assisted extraction for 1 minute was estimated before and after enzyme treatment. It was realised that macerating gave better result than cellulase enzyme. The graph result shows released total polyphenols in gallic acid and catechin equivalence i.e. GAE & CE. It was observed and analysed that there was a rise of 1.14 and 1.15 fold in cellulase and 1.32 and 1.35 fold after macerating enzyme treatment respectively. Total flavonoids in equivalence of quercetin and rutin trihydrate i.e. QE & RTE, it was also seen that there was rise of 4.69 and 0.92 fold in cellulase and 3.18 and 2.56 fold after macerating enzyme treatment respectively; while total anti-oxidant activity in ascorbic acid equivalence (AAE) terms was risen by 1.23 fold in cellulase and 1.67 in macerating enzyme respectively. There was a significant effect of enzyme on plant polysaccharide as the amounts of released polyphenols and flavonoids after enzymes treatment were quite notable. SPSS ANOVA coding states that cellulase enzyme treatment resulted highest release of flavonoids in quercetin equivalence whereas macerating enzyme showed highest polyphenol release in GAE & CE, as well as the total antioxidant activity (AAE).

Catechin inhibitory effect is well documented in literature but during the analysis there it was observed that the lower amount of catechin presence resulted in negative effect during the concentration range of 25 mcg to 75 mcg. However, when the concentration of catechin was increased to 100 mcg to 300 mcg it favoured and facilitates the reaction by showing positive increase in product amount. Hence, presence of catechin in lower amount inhibits the reaction while higher amount facilitates the enzyme activity.

ACKNOWLEDGEMENTS

The authors would like to thank DBT, Government of India for the financial support provided to the Department of Botany, Ramniranjan Jhunjhunwala College under the DBT-Star College Scheme.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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