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Modified bicinchoninic acid assay for accurate determination of variable length reducing sugars in carbohydrates

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Abstract

The accurate determination of reducing ends of malto-oligosaccharides is essential for calculating the enzyme activities of starch debranching enzymes. The suitability of the 3,5-Dinitrosalicylic acid (DNS) method, the Dygert method, and the Bicinchoninic acid (BCA) method for accurate determination of reducing ends from malto-oligosaccharides of different chain lengths is compared. The results showed that BCA assay was much more accurate than the other assays. The results for the BCA assay showed that different malto-oligosaccharides gave observed (measured) values that were significantly similar to the expected (predetermined) values. In contrast, the DNS and Dygert assays underestimated the amount of reducing sugar present for glucose. Furthermore, both DNS and Dygert methods showed increasing degree of overestimation of the amount of reducing sugar present with the increasing length of the malto-oligosaccharide sugar chains. The BCA assay can suitably quantify reducing sugars even in mixtures of oligosaccharides with different chain lengths. Thus, enzyme activities can be measured without bias towards higher values for enzymes that preferentially cleave the longer chain lengths.

Introduction

Linear malto-oligosaccharides with various chain length are products of hydrolysis of starch by several starch debranching enzymes such as isoamylase and pullulanase. Starch debranching enzymes play an important role in the synthesis of amylopectin (Zeeman *et al*., 2010). They hydrolyse the α-1,6-branches in glucan (Hussain *et al*., 2003), producing linear malto-oligosaccharides (González, 1994). The accurate determination of reducing ends of these malto-oligosaccharides is essential for calculating enzyme activity of debranching enzymes. Among the current methods used to quantify reducing ends of sugars are the 3,5-Dinitrosalicylic acid (DNS) method, the Dygert method, and the Bicinchoninic acid (BCA) method. However, enzyme activities may be over or under represented depending on the chain length of the malto-oligosaccharides measured. It has been reported that DNS reducing sugar assay results may have been misinterpreted in literature as it shows differential behaviour for sugars of different chain lengths (Saqib and Whitney, 2011; McIntyre *et al.*, 2013).

The DNS method, which is among the most common methods to quantify reducing sugars (Miller, 1959; Bailey *et al.*, 1992; Gonçalves *et al.*, 2010; Silveira *et al*., 2014), involves the reduction of a nitro group (NO_2) in 3,5 dinitrosalicylic acid to an amine group (NH_2), in alkaline condition, producing 3-amino-5-nitrosalicylic acid. The reaction requires an aldehyde group (CHO) present in the reducing agent (i.e. DTT and reducing sugars), which is oxidised to a carboxyl group (COOH). This reduction produces a coloured solution, which is stabilised by tartrate ions (Miller, 1959). The colour intensity can be measured at 540 nm.

The Dygert method is another method used to measure reducing sugar (Dygert *et al*., 1965; Han *et al.,* 2012; Kotake *et al*., 2013). The assay is based on the reduction of ion Cu^{2+} to Cu^{+} by reducing sugars. The subsequent reaction of ion Cu^{+} and neocuproine in alkaline condition forms a coloured complex that can be read at 405 nm (Dygert *et al.,* 1965).

The BCA method is mainly used in protein quantification (Smith *et al*., 1985; Horn and Eijsink, 2004; Huang *et al*., 2010) but has also been adapted to measure reducing sugars (Hussain *et al.*, 2003; Utsumi *et al*., 2009; Nadour *et al.*, 2015). The reaction with reducing agents results in the reduction of Cu^{2+} to Cu^{+}, followed by the chelation of one Cu^{+} with two BCA molecules, forming an intense

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