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Effect of sucralose on the enzyme kinetics of invertase using real-time NMR spectroscopy and progress curve analysis

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ABSTRACT

Sucralose, a derivative of sucrose, is widely used in noncaloric artificial sweeteners (NAS). Contrary to the belief that sucralose is physiologically inert and a healthy alternative sweetener to natural sugar, emerging studies indicate that sucralose alters the host metabolism as well as the composition of the microbiome. In this manuscript, we use real-time nuclear magnetic resonance (NMR) spectroscopy to demonstrate that sucralose alters the enzymatic conversion of sucrose to glucose and fructose. The real-time NMR progress curve analysis suggests that sucralose has the characteristic of a competitive inhibitor on the kinetics of the enzymatic process. This affects the rate of glucose production, and thus indirectly affecting the mutarotation process of α-D-glucose to β-D-glucose conversion. At a 1:2 M ratio of sucrose to sucralose, the results show that the catalytic efficiency of the enzyme is reduced by more than 50% in comparison to the measurements without sucralose. Altogether, as sucralose alters the rate of glucose production, sucralose cannot be considered inert to the metabolism as several downstream events in both prokaryotic and eukaryotic systems strongly depend on the rate of glucose metabolism.

1. Introduction

Non-caloric artificial sweeteners (NAS) are used as sugar substitutes that provide a sweet taste without added calories or glycemic effects in dietary products. Popular NAS use the sucrose derivative *1, 6-Dichloro-1, 6-dideoxy-b-D-fructofuranosyl-4-chloro-4-deoxy-a-D-galactopyranoside*, commonly known as sucralose, a disaccharide that has a similar structure to sucrose [1,2]. The difference between the structures are three of the hydroxyl groups, at position C4, C1′, and C6' (atom labeling follows the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature [3]), are replaced with chlorine atoms in sucralose. Sucralose is thermally stable up to 119 °C in its pure form, is stable throughout a wide pH range (~pH 3–7), approximately 650 times sweeter than sucrose, and does not decompose in long term storage [1,4–6]. These characteristics make sucralose attractive to the food market as an artificial sweetener additive.

Due to these attractive natures of sucralose, many studies have investigated the effect of sucralose intake in humans and organisms. Sucralose has been shown to alter glycemic and insulin responses, which are parts of the glucose metabolic process following glucose ingestion [7]. A study done by Chia et al. showed that individuals using sucralose, and other NAS, resulted in weight gain/obesity, which is the opposite of what users are expecting [8]. Omran et al. showed that the environmental microbial population is incapable of breaking down sucralose, and further suggested that sucralose has the characteristics of a competitive inhibitor during the hydrolysis of sucrose via invertase [9]. Other recent studies have indicated that sucralose activates an ERK1/2-ribosomal protein S6 signaling axis in MIN6 cells (pancreatic β cell lines for glucose metabolism and glucose-stimulated insulin secretion) [10], increases antimicrobial resistance [11], and suppresses food intake by inducing a fasting response in fruit flies [12,13].

The enzymatic conversion of sucrose to glucose and fructose via invertase is one of the most important enzymatic reactions in prokaryotic and eukaryotic cells. This enzyme catalyzed reaction follows the classic Michaelis-Menten model, a well-studied system [14]. Recently, we have implemented an experimental method to monitor the hydrolysis of sucrose via invertase using nuclear magnetic resonance (NMR) spectroscopy, since the chemical shift of the substrate (sucrose) is well defined from the product (glucose)

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[15,16]. This approach allows the conversion of sucrose to glucose (α-D-glucose) and fructose via invertase to be monitored in real-time, and the subsequent mutarotation process that converts α-D-glucose to β-D-glucose. In this manuscript, we extend this procedure to demonstrate the role of sucralose in altering the Michaelis-Menten kinetics of sucrose hydrolysis via invertase. The combination of real-time NMR measurements and progress curve analysis is used to determine the Michaelis-Menten constant (K_M) and maximum velocity (V_{max}). The results from the progress curve analysis indicate that sucralose has the characteristic of a competitive inhibitor to invertase, which significantly reduces the catalytic efficiency of the enzyme.

2. Materials and method

Invertase (EC 3.2.1.26, β-fructofuranosidase, *S. cerevisiae*) was purchased from Sigma-Aldrich with a specific activity of >300 u/mg of solid (pH 4.6, 303 K). Sucrose, D-(+)-Glucose, D_2O (99.9 atom % D) and 3-(Trimethylsilyl) propionic-2, 2, 3, 3-d_4 acid sodium salt (TSP) were also purchased from Sigma-Aldrich. Sucralose was purchased from Alfa Aesar.

2.1. Sample preparation

For all the NMR kinetics experiments, pH 5.0 was used. The sucrose (80 mM) and invertase (5 μg/mL) concentrations were kept constant, while the sucralose concentrations varied. The sucralose concentrations used were 2 mM, 10 mM, 20 mM, 40 mM, 80 mM, 120 mM, and 160 mM. A timer was set at the beginning of the addition of the invertase solution to account for the delay time (the time before the collection of the NMR spectra).

2.2. Real-time NMR measurements of the hydrolysis of sucrose via invertase

A Varian-Agilent 400 MHz NMR spectrometer was used to collect the NMR data. Each 1D-proton NMR spectrum was collected with a spectral width of 14.88 ppm over 32768 number of points (with Ernst angle [17]). Each spectrum was signal averages over 24 transients and 1s relaxation delay between the transients leading to a total time of 90.0 s per experiment (24 × 3.75 s per transient). The 1D-proton NMR spectra were collected in an array fashion one after another and were collected as necessary to follow the reaction to completion. The NMR data were processed using the program MNova NMR™. The area under the curve of the sucrose resonance (at 5.41 ppm), alpha-D-glucose and beta-D-glucose resonance (at 5.22 ppm and 4.64 ppm), and sucralose resonance (at 5.49 ppm) were used to calculate their concentration using TSP (at 0 ppm) as the standard. Progress curve analysis was done following the procedure in the following reference [16].

2.3. Progress curve analysis of the real-time NMR data

The catalyzed breakdown, or hydrolysis, of sucrose, can be shown by the Michaelis-Menten equation. Schnell and Mendoza [22] presented the integrated form of the Michaelis-Menten equation using the Lambert-W function with an application developed by Goudar and co-workers [18,19]. A brief description of enzyme kinetics is given here following our earlier work [16]:

The Michaelis–Menten equation in the differential form can be used to describe the dynamics of substrate depletion as Equation [1]:

$$v = \frac{d[P]}{dt} = -\frac{d[S]}{dt} = \frac{V_{max}[S]}{K_M + [S]} \quad [1]$$

Where [S] is the substrate concentration, V_{max} is the maximal rate of enzymatic turnover (sucrose to glucose and fructose), and K_M represents the Michaelis-Menten half-saturation constant. The first-order differential equation (Equation [1]) can be integrated to obtain the integral form of the Michaelis–Menten equation [20–22] as shown in Equation [2]:

$$K_M \ln\left(\frac{[S]_0}{[S]}\right) + [S]_0 - [S] = V_{max}t \quad [2]$$

The Lambert-W function is a mathematical function, in the form of an exponential function, and has several applications in computer science, mathematics, and physical sciences [23,24]. Mathematically, the exponential function and the natural logarithmic function, ln(x), are exponentially related. Similarly, W*(x)* is defined as the inverse of the function satisfying $ye^y = x$ and its solution expressed by the Lambert-W(x) function like $y = W(x)$.

By substituting $y = [S]/K_M$ in Equation [2] and rearranging, we get Equation [3]:

$$\begin{aligned} ye^y = x(t) &= \exp\left(\frac{[S]_0 - V_{max}t}{K_M} + \ln\left(\frac{[S]_0}{K_M}\right)\right) \\ &= \frac{[S]_0}{K_M}\exp\left(\frac{[S]_0 - V_{max}t}{K_M}\right) \end{aligned} \quad [3]$$

The left-hand side of Equation [3] is analogous to the Lambert-W function in the Corless et al., 1996 article [24]. Thus, using the definition of the Lambert-W function (y = W(x)), an expression for y can be obtained as that expressed in Equation [4]:

$$y = W\left\{\frac{[S]_0}{K_M}\exp\left([S]_0 - V_{max}t/K_M\right)\right\} \quad [4]$$

Further substituting $y = [S]/K_M$ back in Equation [4], we get Equation [5]:

$$[S] = K_M W\left\{\frac{[S]_o}{K_M}\exp\left(\frac{[S]_o - V_{max}t}{K_M}\right)\right\} \quad [5]$$

Equation [5] relates the substrate concentration at any time ([S]) to its initial concentration ($[S]_0$), the Michaelis–Menten kinetic parameters V_{max} and K_M. Equation [5] is used to fit the real-time experimental data obtained for enzyme kinetics using the analysis code written in the R-Statistical environment [25].

3. Results

3.1. NMR spectral differentiation between sucrose, glucose, and sucralose

Glucose, sucrose, and sucralose all have at least one proton with a distinct resonance, granting the ability to perform real-time analysis of the enzyme kinetics via NMR spectroscopy. Fig. 1 shows the NMR spectra of sucrose (Fig. 1a), sucralose (Fig. 1b) and glucose (Fig. 1c). The C1 proton for sucrose shows a double peak (doublet) close to the resonance frequency of 5.41 ppm, while the same proton in the sucralose is shifted downfield to 5.49 ppm. Also, the C5 and C4 protons of sucralose resonate at 4.42 ppm and 4.54 ppm, respectively (Fig. 1b). As shown in Fig. 1c, the NMR spectrum for glucose depicts a doublet at 5.22 ppm (C1 proton) [26] for α-D-glucose and the second doublet at 4.64 ppm (C1 proton) for β-D-glucose. The relative intensities of the α-D-glucose and β-D-glucose peaks (Fig. 1c) are dependent on the thermodynamic