Carbohydrate Research 455 (2018) 5–9

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

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

Cheenou Her [a], Jaideep Singh [a], V.V. Krishnan [a, b, *]

[a] Department of Chemistry, California State University, Fresno CA 93740, United States
[b] Department of Pathology and Laboratory Medicine, School of Medicine, University of California, Davis CA 95616, United States

ARTICLE INFO

Article history:
Received 4 July 2017
Received in revised form
19 October 2017
Accepted 27 October 2017
Available online 31 October 2017

Keywords:
Enzyme kinetics
NMR spectroscopy
Progress curve analysis
Sucralose
Sucrose

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)

* Corresponding author. Department of Chemistry, California State University, Fresno CA 93740, United States
E-mail addresses: krish@csufresno.edu, vvkrishnan@ucdavis.edu (V.V. Krishnan).

https://doi.org/10.1016/j.carres.2017.10.019
0008-6215/

[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

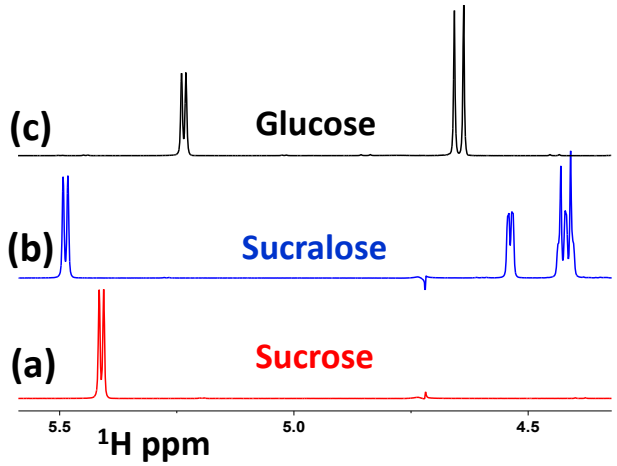

Fig. 1. Differentiation of sucrose, glucose, and sucralose nuclear magnetic resonance (NMR) spectra in the absence of enzymatic activity. (a) Expanded region of the one-dimensional NMR spectrum of sucrose the substrate. (b) Expanded region of the NMR spectrum of sucralose, where the peak has shifted downfield due to change in the chemical structure. (c) Expanded region of the NMR spectrum of glucose showing both the α- (downfield) and β- (up-field) anomers at their ambient temperature equilibrium populations.

equilibria of the glucose anomers in solution. At ambient temperature, the equilibrium favors the existence of the more stable β-D-glucose anomer (~64%), as depicted by the higher peak intensity in Fig. 1c [27]. The distinct, well-resolved peaks of sucrose, sucralose, and glucose enable the differentiation between molecules in the same sample, allowing for the determination of kinetic rates of conversion by the enzyme catalysis.

3.2. Real-time enzymatic conversion of sucrose to glucose

With the known concentration of the internal reference (TSP), the integration of the area under the respective peaks of sucrose ([S]), glucose ([P]) and sucralose allowed for the determination of the rate of change of the reactant and product concentrations (Fig. 2). Fig. 2a illustrates the plot of the real-time hydrolysis of the sucrose ([S]) via invertase, without sucralose (filled black circle) and in the presence of sucralose (filled red squares). While the hydrolysis of sucrose occurred in both conditions, the concentration of sucralose (blue diamonds) remained constant in the presence of invertase.

Upon creation of α-D-glucose ($[P]_\alpha$) from sucrose, the interaction with water leads to the mutarotation of α-D-glucose to β-D-glucose ($[P]_\beta$), as shown in Fig. 2b, until an equilibrium is reached between α- and β-anomers of glucose. This thermodynamic equilibrium between both glucose anomers is temperature-dependent and is the reason the concentration profile of α-D-glucose decreases as the β-D-glucose anomer is formed at room temperature (Fig. 2b) [27]. Mutarotation of anomers are inherent to carbohydrates, and most of the carbohydrates exist in multiple anomeric forms (predominantly in α and β-anomers in the case of glucose). However, the co-existence of these multiple anomeric forms is due to solvation effects and is independent of the enzyme action.

The current experimental parameters are chosen such that there is enough time-resolution particularly at the early time points to efficiently determine the enzyme kinetics and the S/N required analyzing the data. Furthermore, the kinetic measurements focus more on the rates (decay of sucralose or growth of glucose) rather than the absolute concentration; we assume that shorter relaxation delay (3.75 s per transient) with the Ernst angle would not bias the results presented here.

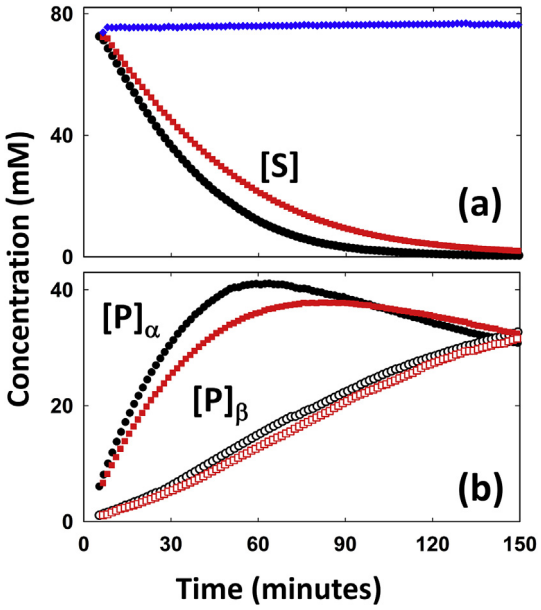

Fig. 2. Real-time qNMR results of substrate depletion ([S]) and product formation ($[P]_\alpha$). The concentration of sucrose (panel a, black symbols) and glucose (panel b, black symbols) is shown as a function of time due to the hydrolysis process of the enzyme invertase. Anomerization of α to the β anomer of glucose is also observed, depicting the dynamic equilibrium of the mutarotation process. In the presence of sucralose, both the substrate (red symbols, panel a) and product (red symbols, panel b) are altered. The concentration of sucralose does not change during the reaction (blue symbols) but alters the rate of hydrolysis of sucrose via invertase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Effect of sucralose of the enzymatic rate of conversion of sucrose

Though the sucralose concentration remains constant during the experiment (blue diamond symbols Fig. 2a), the presence of sucralose significantly alters the rate at which sucrose is enzymatically hydrolyzed via invertase. The enzymatic conversion of sucrose to glucose and fructose follows the standard Michaelis-Menten enzyme kinetics mechanism [28–30]. The enzymatic reaction can be monitored either regarding the substrate (sucrose) or terms of the product (glucose) concentration. The characteristic of the enzyme action, in the absence of any inhibitory process, is measured by the Michaelis-Menten parameters defined by the Michaelis-Menten constant (K_M) and maximal velocity (V_{max}).

Using the Lambert-W function, described in equation [5], to fit the sucrose progress curve on time, the Michaelis-Menten constant and maximum velocity can be determined directly from a single NMR experiment [16]. For pure enzyme kinetics (no sucralose), the direct method yields a K_M of 69.21 mM ± 1.24 mM and V_{max} of 3.52 ± 0.04 mM/min. As shown in Fig. 2a, sucralose slows down the conversion of sucrose to α-D-glucose. In the presence of sucralose, the direct method yields a K_M of 116.81 mM ± 2.09 mM and V_{max} of and 3.78 mM/min ±0.05 mM/min. Progress curve analysis produces fairly consistent results with an average coefficient variation (coefficient variation (CV) in %) of K_M measured from the three trials is 9%, and that of the V_{max} is 7% [16].

3.4. The effect of sucralose at varying concentration on the enzymatic conversion of sucrose

The presence of sucralose, even at lower concentrations, affects the catalytic mechanism of invertase. Fig. 3 shows the

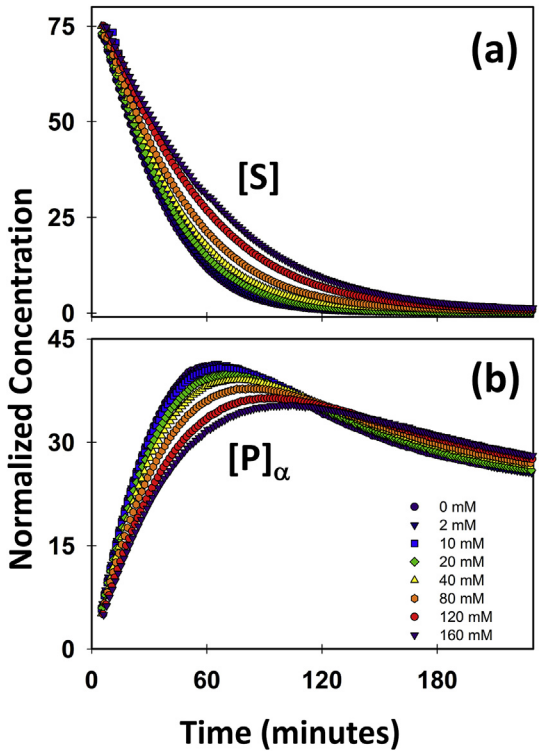

Fig. 3. The hydrolysis of sucrose via invertase in the presence of varying sucralose concentration. (a) The rate of hydrolysis of sucrose and (b) rate of formation of α-D-glucose as a function of varying the concentration of sucralose. The sucralose concentrations are noted in the legends.

concentration-dependent effect of sucralose on invertase hydrolyzing sucrose into α-D-glucose ($[P]_\alpha$) and fructose (not shown). As shown in Fig. 3 the concentrations of sucralose studied were 0 mM (no sucralose), 2 mM, 10 mM 20 mM, 40 mM, 80 mM, 120 mM, and 160 mM. Table 1 shows the enzyme's kinetic parameters estimated, from the progress curve analysis. The higher the concentration of sucralose present during the reaction, the slower the hydrolysis rate of sucrose. The presence of sucralose also had an indirect consequence. Since the rate of formation of α-D-glucose was altered, the mutarotation rate of α-D-glucose to β-D-glucose was also affected (data not shown).

The real-time NMR-based progress curve analysis demonstrates the deleterious effects of sucralose in the overall enzyme kinetics process. Using the definition by Koshland [31], the catalytic efficiency of the enzyme activity is determined (Fig. 4). Catalytic efficiency provides insight into enzymatic mechanisms and the functional effects of sucralose concentrations on activity. The roles of many enzymes in a biological system, which relates to the chemical kinetics and equilibrium processes, are essential for the overall function in many critical biochemical reactions. The overall

Table 1
Effect of sucralose on the Michaelis-Menten kinetics using direct NMR method.

Sucralose (mM)	Sucrose: Sucralose	K_M (mM)	V_{max} (mM/min)
0	1:0	69.21 ± 1.24	3.52 ± 0.04
2	4:1	74.61 ± 1.31	3.62 ± 0.04
10	8:1	94.13 ± 2.06	4.38 ± 0.07
20	4:1	87.28 ± 1.41	3.75 ± 0.02
40	2:1	100.60 ± 1.71	3.86 ± 0.05
80	1:1	116.81 ± 2.09	3.78 ± 0.05
120	1:1.5	129.80 ± 2.68	3.51 ± 0.05
160	1:2	155.10 ± 4.58	3.54 ± 0.08

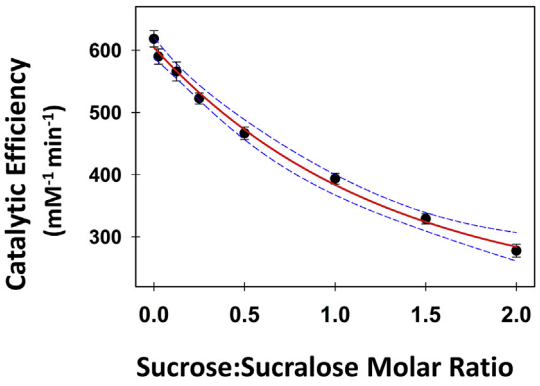

Fig. 4. Sucralose concentration effect on enzymatic activity. Variation of catalytic efficiency (CE) as a function of the molar ratio of sucralose to sucrose concentration. With increasing concentration of sucralose, the effectiveness of the enzymatic reaction drops significantly. The continuous red line shows the exponential regression and the dotted blue lines shows the 95% confidence interval of the fit. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

efficiency of the enzyme function is significantly decreased, more or less in an exponential fashion with increasing concentration of sucralose. An exponential fit (Fig. 4), suggests that the catalytic efficiency (in units of mM^{-1} min^{-1}) reduces at a rate of decreases by 50% for every increase in the molar ratio of sucralose to sucrose by 0.87 ± 0.15.

4. Discussion

The first step in the metabolism of sucrose is the conversion to simpler, soluble monosaccharide forms (glucose and fructose). Glucose (or fructose) can be transported across the intestinal wall and delivered to the tissues, but this transportation process depends primarily on the production rate of glucose (or fructose) by enzyme kinetics. The rate of metabolic flow (also known as flux) through the homeostatic mechanisms pathway is high, but the concentration of substrate/intermediates/products remains constant. If this steady state is disrupted by an external change on the rate of glucose formation, the pathway will change, and regulatory mechanisms will be triggered. This will cause the organism to try arriving at a new steady state to achieve homeostasis, the complex dynamics between the glucose and other elements in the cell that maintain a relatively constant blood sugar level [32]. Therefore, from a simple coupled reaction point of view, the rate of glucose production is expected to affect the energy homeostasis and the rest of the downstream pathways related to glucose metabolism.

In the presence of sucralose, the enzyme kinetics of invertase and sucrose was altered, thus affecting the production rate of glucose. This effect on the early stages of sugar metabolism could result in a cascade of downstream effects since the presence of glucose in the blood triggers the release of insulin. Insulin is important in regulating many other pathways that allow for the distribution of glucose to muscle and fat cells via glucose transporters. The level of insulin, which is triggered by the presence of glucose in the blood, also plays a major role in determining whether the glucose could be stored as glycogen, an energy storage in the cell. When the cells need additional energy (glucose) the hormone glucagon is released to signal the breakdown of glycogen to glucose in the blood.

The physical meaning of K_M (concentration units) represents the state when half the active sites of the enzyme are occupied by the substrate. When the enzyme has a low affinity for the substrate, a higher concentration of substrate molecules is required to saturate

half the active sights leading to a high K_M value. On the other hand, if the enzyme has a higher affinity to the substrate then the substrate does not have to occupy a large number of active sites, a small number will suffice to lead to a low K_M value. The increase in the K_M values with an increase in the sucralose concentration suggests reduced affinity to the substrate, sucrose, due to the presence of sucralose. The maximal velocity, V_{max} represents how fast the enzyme can catalyze the reaction-the number of substrate molecules being catalyzed per unit time. The average value of the V_{max} was not altered significantly (3.74 ± 0.27 mM/min, Table 1), suggesting that sucralose would not change the catalytic function of the enzyme. The catalytic efficiency (Fig. 4) drops as much as 50% for every 0.87 ± 0.15 equimolar increase of sucralose on sucrose (based on exponential regression).

The trend of increasing K_M and constant V_{max}, with increasing sucralose concentration, is one indication that sucralose may be a competitive inhibitor. The molecular structure of sucralose gives insight into how it interacts with the active site of invertase, facilitating competitive inhibition of sucrose hydrolysis. Sucralose has a similar structure to sucrose, giving it the potential to bind to the active site of invertase; however, hydrolysis does not occur. This is believed to be due to the negative charge repulsion between the carboxylate anion in the active site of invertase with the chlorine atoms on sucralose [33]. The hydrogen bonding of the three hydroxyl groups at the C4, C1′, and C6′ positions of sucrose are crucial for the specificity and recognition of the sucrose by invertase [34]. Since the three-hydroxyl groups of sucralose are replaced with chlorine atoms, this results in the loss of hydrogen bonding that is crucial in the stabilization of the intermediate complex. The competitive nature of sucralose with the active site of the enzyme, combined with its inability to be hydrolyzed fits the model of a competitive inhibitor.

Concluding from the data obtained, sucralose has the characteristic of a competitive inhibitor on the hydrolysis of sucrose via invertase. The initial interaction of sucralose with invertase enzyme, as witnessed by the indirect effect of sucralose on the mutarotation process, shows sucralose can have potentially hazardous effects on downstream processes in glucose metabolism. While sucralose cannot be metabolized, the potential to directly or indirectly alter “normal” reaction rates that are crucial for biological systems calls for caution when using sucralose as an alternative to natural sugars.

Acknowledgements

The authors thank C. Cortney for critical reading of the manuscript. CH and JS were in part supported by Graduate Net Initiative (Fresno State), and CH was supported in part by a graduate fellowship from National Science Foundation (NSF Award # 1059994).

References

[1] I. Knight, The development and applications of sucralose, a new high-intensity sweetener, Can. J. Physiology Pharmacol. 72 (4) (1994) 435–439.
[2] O.A.A. AlDeeb, H. Mahgoub, N.H. Foda, in: G.B. Harry (Ed.), Chapter Ten - Sucralose, in Profiles of Drug Substances, Excipients and Related Methodology, Academic Press, 2013, pp. 423–462.
[3] A.D. McNaught, International union of pure and applied chemistry and international union of biochemistry and molecular biology. Joint commission on biochemical nomenclature. Nomenclature of carbohydrates, Carbohydr. Res. 297 (1) (1997) 1–92.
[4] G. Bannach, R.R. Almeida, L.G. Lacerda, E. Schnitzler, M. Ionashiro, Thermal stability and thermal decomposition of sucralose, Eclética Quím. 34 (2009) 21–26.
[5] D.N. de Oliveira, M. de Menezes, R.R. Catharino, Thermal degradation of sucralose: a combination of analytical methods to determine stability and chlorinated byproducts, Sci. Rep. 5 (2015) 9598.
[6] M.E. Quinlan, M.F. Jenner, Analysis and stability of the sweetener sucralose in beverages, J. Food Sci. 55 (1) (1990) 244–246.
[7] M.Y. Pepino, C.D. Tiemann, B.W. Patterson, B.M. Wice, S. Klein, Sucralose affects glycemic and hormonal responses to an oral glucose load, Diabetes Care 36 (9) (2013) 2530–2535.
[8] C.W. Chia, M. Shardell, T. Tanaka, D.D. Liu, K.S. Gravenstein, E.M. Simonsick, J.M. Egan, L. Ferrucci, Chronic low-calorie sweetener use and risk of abdominal obesity among older adults: a cohort study, PLoS One 11 (11) (2016) e0167241.
[9] A. Omran, G. Ahearn, D. Bowers, J. Swenson, C. Coughlin, Metabolic effects of sucralose on environmental bacteria, J. Toxicol. 2013 (2013) 6.
[10] M.L. Guerra, M.A. Kalwat, K. McGlynn, M.H. Cobb, Sucralose activates an ERK1/2-ribosomal protein S6 signaling axis, FEBS Open Bio 7 (2) (2017) 174–186.
[11] Y. Qu, R. Li, M. Jiang, X. Wang, Sucralose increases antimicrobial resistance and stimulates recovery of Escherichia coli mutants, Curr. Microbiol. (2017) 1–4.
[12] J.H. Park, G.B. Carvalho, K.R. Murphy, M.R. Ehrlich, W.W. Ja, Sucralose suppresses food intake, Cell Metab. 25 (3) (2017) 484–485.
[13] Q.-P. Wang, Yong Q. Lin, L. Zhang, Yana A. Wilson, Lisa J. Oyston, J. Cotterell, Y. Qi, Thang M. Khuong, N. Bakhshi, Y. Planchenault, Duncan T. Browman, Man T. Lau, Tiffany A. Cole, Adam C.N. Wong, Stephen J. Simpson, Adam R. Cole, Josef M. Penninger, H. Herzog, G.G. Neely, Sucralose promotes food intake through NPY and a neuronal fasting response, Cell Metab. 24 (1) (2016) 75–90.
[14] K.A. Johnson, R.S. Goody, The original Michaelis constant: translation of the 1913 michaelis-menten paper, Biochemistry 50 (39) (2011) 8264–8269.
[15] F. Exnowitz, B. Meyer, T. Hackl, NMR for direct determination of Km and Vmax of enzyme reactions based on the Lambert W function-analysis of progress curves, Biochim. Biophys. Acta, Proteins Proteomics 1824 (3) (2012) 443–449.
[16] C. Her, A.P. Alonzo, J.Y. Vang, E. Torres, V.V. Krishnan, Real-time enzyme kinetics by quantitative NMR spectroscopy and determination of the michaelis–menten constant using the Lambert-W function, J. Chem. Educ. 92 (11) (2015) 1943–1948.
[17] R.R. Ernst, W.A. Anderson, Application of fourier transform spectroscopy to magnetic resonance, Rev. Sci. Instrum. 37 (1) (1966) 93–102.
[18] C.T. Goudar, S.K. Harris, M.J. McInerney, J.M. Suflita, Progress curve analysis for enzyme and microbial kinetic reactions using explicit solutions based on the Lambert W function, J. Microbiol. Methods 59 (3) (2004) 317–326.
[19] C.T. Goudar, J.R. Sonnad, R.G. Duggleby, Parameter estimation using a direct solution of the integrated Michaelis-Menten equation, Biochim. Biophys. Acta 1429 (2) (1999) 377–383.
[20] R.M.F. Bezerra, A.A. Dias, Utilization of integrated Michaelis-Menten equation to determine kinetic constants, Biochem. Mol. Biol. Educ. 35 (2) (2007) 145–150.
[21] R.G. Duggleby, Quantitative analysis of the time courses of enzyme-catalyzed reactions, Methods 24 (2) (2001) 168–174.
[22] S. Schnell, C. Mendoza, Closed form solution for time-dependent enzyme kinetics, J. Theor. Biol. 187 (2) (1997) 207–212.
[23] D.A. Barry, J.Y. Parlange, L. Li, H. Prommer, C.J. Cunningham, E. Stagnitti, Analytical approximations for real values of the Lambert W-function, Math. Comput. Simul. 53 (1–2) (2000) 95–103.
[24] R.M. Corless, G.H. Gonnet, D.E.G. Hare, D.J. Jeffrey, D.E. Knuth, On the Lambert W function, Adv. Comput. Math. 5 (4) (1996) 329–359.
[25] R Core Team, R: a Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2014.
[26] J.E. Gurst, NMR and the structure of D-glucose, J. Chem. Educ. 68 (12) (1991) 1003.
[27] F. Franks, P.J. Lillford, G. Robinson, Isomeric equilibria of monosaccharides in solution - influence of solvent and temperature, J. Chem. Society-Faraday Trans. I 85 (1989) 2417–2426.
[28] L. Michaelis, M.L. Menten, Die kinetik der invertinwirkung, Biochem. z 49 (333–369) (1913) 352.
[29] G.E. Briggs, J.B.S. Haldane, A note on the kinetics of enzyme action, Biochem. J. 19 (2) (1925) 338–339.
[30] H. Lineweaver, D. Burk, The determination of enzyme dissociation constants, J. Am. Chem. Soc. 56 (3) (1934) 658–666.
[31] D.E. Koshland Jr., The application and usefulness of the ratio kcat/KM, Bioorg. Chem. 30 (3) (2002) 211–213.
[32] J.S. Bogan, Regulation of glucose transporter translocation in health and diabetes, Annu. Rev. Biochem. 81 (2012) 507–532.
[33] S. Shall, A.B.A. Waheed, The mechanism of action of yeast invertase, Biochem. J. 122 (1) (1971) 19.
[34] C.L. Thomas, Is sucralose too good to be true? Biochem. Mol. Biol. Educ. 40 (1) (2012) 14–18.