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Inhibition of porcine small intestinal sucrase by valienamine

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Abstract

Valienamine, an aminocyclitol, has been isolated from the enzymolysis broth of validamycins. The absolute configuration of valienamine is similar to that of α-D-glucose. The inhibitory effect of this amino-sugar analog of α-D-glucose, valienamine, on porcine small intestinal sucrase was examined. Valienamine was found to be potent, competitive reversible inhibitor of porcine small intestinal sucrase *in vitro* with an IC_{50} value of 1.17×10^{-3} M. Valienamine also exhibited dose-dependent, instantaneous inhibition of porcine small intestinal sucrase. The inhibition of porcine small intestinal sucrase by valienamine was pH-independent.

Keywords: *Valienamine, porcine small intestinal sucrase, enzyme inhibition, validamycins*

Introduction

Valienamine, [(1*S*, 2*S*, 3*S*, 4*R*)-1-amino-5-(hydroxymethyl) cyclohex-5-ene-2, 3, 4-triol], was first isolated from the microbial degradation of validoxylamine A by *Pseudomonas denitrificans* [1,2]. Later, it was prepared from the degradation of validoxylamine A by *Flavobacterium saccharophilum* [3] or with the N-bromosuccinimide (NBS) cleavage of validoxylamine A or its derivatives [4,5]. The absolute configuration of valienamine is similar to that of α-D-glucose (Figure 1) [6]. Thus, it demonstrates powerful glycosidase inhibition activity [6,7]. Glycosidases are enzymes for the cleavage of glycosidic bonds and are responsible for glycoprotein processing on the surface of the cell wall and for carbohydrate digestion in animals. Inhibition of these enzymes has significant implications for both antiviral and antidiabetic chemotherapy. Plasma levels of D-glucose and insulin are usually high in diabetics, especially after food ingestion. Limiting intestinal digestion of dietary carbohydrates by inhibition of intestinal α-glucosidases has been suggested as a possible means of controlling diabetes and obesity. Thus, α-D-glucosidase inhibitors are thought to be valuable aids in the treatment of diabetes and obesity. They act by delaying the absorption of carbohydrates, thereby inhibiting postprandial hyperglycemia and hyperinsulinemia. Furthermore, several studies have confirmed the value of the inhibitors of the processing enzyme glucosidase I in the treatment of cancer, and in inhibiting the human immunodeficiency virus (HIV) replication, the etiologic agent for acquired immune deficiency syndrome (AIDS) and AIDS-related complex. Valienamine is a very important chemical intermediate in the synthesis of other pseudo-oligosaccharidic α-glucosidases inhibitors, such as acarbose [8], adiposins [9], acarviosin [10], trestatins [11], voglibose [12], and so on. These pseudo-oligosaccharides exhibit stronger enzyme inhibition activities than valienamine itself. We have recently reviewed the properties of valienamine and its related analogues [13]. Studies on valienamine have gained more and more attention.

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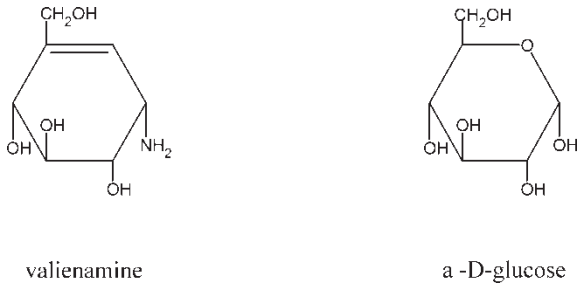

Figure 1. Chemical structures of valienamine and α-D-glucose.

In order to achieve a scale production of valienamine, some strains of microorganisms were isolated from soil in our laboratory, one of which was *Pseudomonas* sp. HZ 519 which is able to degrade validamycin A into valienamine.

Sucrase [EC.3.2.1.26] is an important disaccharidase since it is capable of hydrolyzing sucrose to fructose and glucose monomeric units [14]. The disaccharidase, sucrase accumulates in a variety of organisms including animals, bacteria, yeast, fungi. In higher animals, the sucrase has been found in the brush border membranes of the small intestine where it functions in the hydrolysis of ingested sucrose.

High levels of blood sugar result in diabetes and obesity. Many glycosidase inhibitors can regulate the absorption of carbohydrate. Since the sucrase is involved in intestinal digestion, it is interest of increased that small intestinal sucrase is inhibited by valienamine.

This paper deals with the inhibition of porcine small intestinal sucrase by the amino-sugar analog of glucose, valienamine. The kinetic constants (apparent K_m and V_{max} values) and IC_{50} value (molar concentration required to give 50% inhibition) against porcine small intestinal sucrase were determined.

Materials and methods

Materials

Valienamine was obtained from the enzymolysis broth of validamycin A. Porcine small intestinal sucrase was extracted from porcine small intestine brush border membrane that was bought in a local slaughterhouse. All other chemicals were from local suppliers and were of analytical grade.

Methods

Isolation of valienamine from the enzymolysis broth of validamycins. *Pseudomonas* sp. HZ 519, which was isolated from the rice fields of Hangzhou, China, was found to efficiently decompose validamycin A. The outline of the degradation process is described as follows: (1) validamycin A was first hydrolyzed to validoxylamine A by a glucosidase; (2) the competitive cleavage of two C–N bonds in validoxylamine A led to the formation of valienamine and validamine [3]. For the preparation of valienamine, *pseudomonas* sp. HZ 519 was cultured at 28°C for 7 days on a shaker in a medium at pH 8.0 consisting of validamycin A 1.5%, $(NH_4)_2SO_4$ 0.75%, KCl 0.5%, $Na_2HPO_4{\cdot}12H_2O$ 6.97% and $NaH_2PO_4{\cdot}2H_2O$ 0.16%. The culture broth was passed through a column of Amberlite IRC-50 (NH_4^+ form, 500 mL), which was eluted with 0.5 N aqueous ammonia. The concentrate of the eluate was chromatographed on a column of Dowex 1 × 2 (OH^- form, 500 mL) column and developed with water to give valienamine. Valienamine is soluble in water. The concentration of valienamine was determined by thin-layer chromatography (TLC method) with a TLC Scanner (TLC Scanner 3, Linomat 5, Chromatogram-immersiom device III, TLC sprayer, Camag, Switzerland) in accord with the R_f value of valienamine described by Kameda et al [6].

Preparation of porcine small intestinal sucrase. All procedures for preparation of porcine small intestinal sucrase were performed at 4°C. Porcine small intestinal mucosal was collected by scraping the lumina surface firmly with a spatula. The mucosal scrapings were homogenized with 0.2 M sodium phosphate buffer pH 7.0, and then centrifugated at 12000 r·min^{-1} for 15 min. The supernatant fraction contained porcine small intestinal sucrase. Solid ammonium sulfate was added to the supernatant fraction to a saturated concentration. The salted extract was stirred overnight at 4°C and the precipitate that formed was collected by centrifugation at 15000 r·min^{-1} for 15 min. The precipitate was solubilized in 0.2 M sodium phosphate buffer (pH 7.0) and dialyzed overnight against the same buffer. After dialysis, the concentrated protein was stored at 4°C [15]. Concentrated protein was used in this study on the inhibition of porcine small intestinal sucrase by a amino-sugar analog of α-D-glucose, valienamine.

Assay of porcine small intestinal sucrase. The activity of the porcine small intestinal sucrase was determined by measuring the formation of glucose when the enzyme was incubated with sucrose. The standard incubation mixture contained 0.2 M sodium phosphate buffer (pH 6.6), 0.1 M sucrose and 0.4 ml porcine small intestinal sucrase (about 0.3 U, according to the definition). After incubation at 37°C for 30 min, the liberated glucose was determined by the DNS method [16]. One unit of enzyme activity was defined as the quantity of enzyme producing 1 μmol glucose per min at 37°C pH 6.6. In the inhibition test, the reaction was started by adding porcine small intestinal sucrase with

different activity units and valienamine at different concentrations. After incubation at 37°C for 10 min, 0.1 M sucrose was added. Finally, 0.5 ml of 1 M NaOH was added to the culture tubes to stop the reaction.

The percentage inhibition of porcine small intestinal sucrase was calculated as follows:

$$\text{Percentage (\%) inhibition} = (\text{Ua} - \text{Ue})/\text{Ua} \times 100\%.$$

where Ua represents the initial sucrase activity, Ue represents the final sucrase activity.

Results and discussions

Effects of valienamine on porcine small intestinal sucrase

Valienamine, isolated from the enzymolysis broth of validamycins, was tested as an inhibitor of porcine small intestinal sucrase. In accord with the origin of the enzyme, the influence of pH on porcine small intestinal sucrase activity were examined by varying the pH of 0.2 M sodium phosphate buffer from pH 5.3 to 7.4 in the absence or presence of 2.5 mM valienamine respectively (Figure 2). Incubations were conducted in a final volume of 1.8 ml containing 0.4 ml porcine small intestinal sucrase, 0.1 M sucrose, 0.2 M sodium phosphate buffer, at 37°C for 30 min. In the absence of valienamine, maximum activity was observed at pH 6.6. However, when valienamine was added to the incubations, maximum activity was also observed at pH 6.6. Surprisingly, the shapes of these two curves were very alike. To investigate the cause of this identical result, the extent of the decrease of relative activity at the same pH value was calculated. It showed that the same level of inhibition by valienamine was observed at each pH value. The results indicated that the effects of pH and valienamine on enzyme activity were independent each other. That is to say, the pH value did not affect the inhibitory effect of valienamine on the porcine small intestinal sucrase. Also, adding valienamine did not influence the effect of pH on enzyme activity. The inhibition of porcine small intestinal sucrase by valienamine was pH-independent.

Various concentrations of valienamine were added to assay mixtures containing 0.2 M sodium phosphate buffer of pH 6.6, 0.1 M sucrose, and 0.4 ml (0.3U) porcine small intestinal sucrose, and the incubations were carried out for 30 min at 37°C. Figure 3 shows the effect of increasing concentration of valienamine on the activity of porcine small intestinal sucrase and clearly demonstrates that valienamine is very potent inhibitor of the enzyme. It was found that 50% of inhibition of porcine small intestinal sucrase occurred at a valienamine concentration of about 1.17×10^{-3} M, which gives an IC_{50} value of about 1.17×10^{-3} M. This IC_{50} value was not equal to that mentioned by Kameda *et al.*[6] The possible reason was that the characterization of small intestinal sucrase varies with different pig species and different segments of the small intestine. In the experimental range of valienamine concentration from 0.125 mM to 5 mM, the plot of valienamine concentration vs. inhibition percent was approximately linear demonstrating dose-dependent inhibition of the enzyme.

Valienamine was added at different concentration of 0.125 mM, 0.500 mM, 1.250 mM, 2.500 mM, and 5.000 mM respectively, and mixtures were incubated for the times shown. The progress curves for the inhibition of porcine small intestinal sucrase by different concentrations of valienamine are shown in Figure 4. It is seen that valienamine demonstrated dose-dependent inhibition of porcine intestinal sucrase.

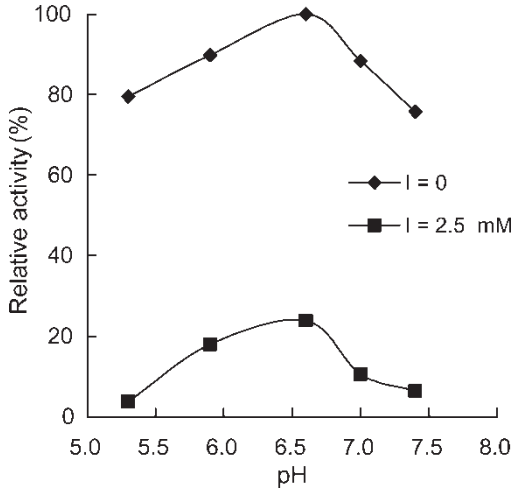

Figure 2. Effects of pH on porcine small intestinal sucrase activity in the absence or presence of 2.5 mM valienamine. The activity of porcine small intestinal sucrase at pH 6.6 was taken as 100%.

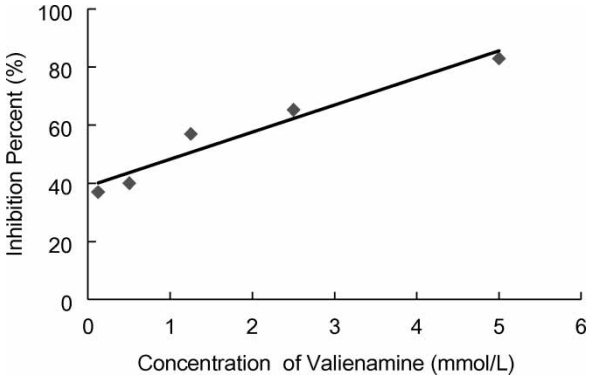

Figure 3. Effects of valienamine concentration on the activity of porcine small intestinal sucrase.

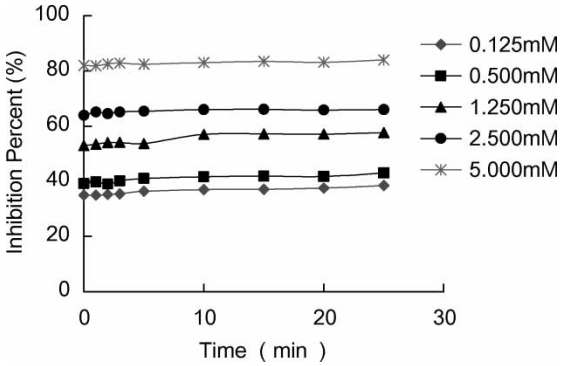

Figure 4. Effects of enzyme/inhibitor pre-incubation times on the activity of porcine small intestinal sucrase.

The interaction of the inhibitor valienamine and porcine small intestinal sucrase is instantaneous since valienamine displayed no time-dependent inhibition of porcine intestinal sucrase within 25 min. This implied that the process of the valienamine combining with porcine small intestinal sucrase was very rapidly.

Kinetics of inhibition of porcine small intestinal sucrase

The double reciprocal Lineweaver-Burk plots showed that valienamine was a competitive inhibitor of porcine small intestinal sucrase (Figure 5). In this experiment, the effect of valienamine on porcine small intestinal sucrase activity was examined. The substrate concentration curves were measured with porcine small intestinal sucrase in the absence or presence of 1.6 mM or 2.5 mM of valienamine respectively. Km value is the indicator of the affinity of an enzyme towards its substrates; the greater the value of Km, the less is the affinity. The K_i value for valienamine was about 7.7×10^{-4} M. This means that, it was approximately less than 100 times the K_m value (5.0×10^{-2} M) of the enzyme. The data suggested that valienamine had about 100 times greater affinity for the active site of the enzyme than its substrate.

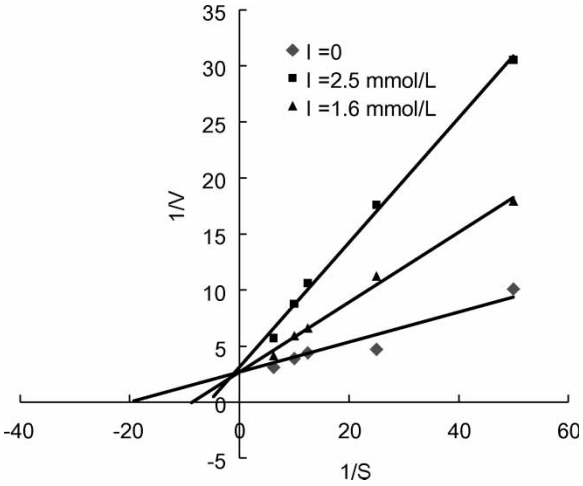

Figure 5. Lineweaver-Burk double reciprocal plots of porcine small intestinal sucrase activity in the absence or presence of various concentration of valienamine (I). 1/V is defined as (μmol of glucose released per minute)$^{-1}$, 1/S was defined as M^{-1}.

The main conclusion to be drawn from this work is that valienamine is a powerful inhibitor of porcine small intestinal sucrase activity. The inhibition of the enzyme by valienamine was pH-independent, the association of valienamine and enzyme reached equilibrium very fast and the inhibitory activity of valienamine was linear with its concentration. Valienamine was a competitive inhibitor from the Lineweaver-Burk double reciprocal plot with an IC_{50} value of 1.17×10^{-3} M.

In summary, it was clear from the present studies that valienamine is a potent, competitive inhibitor of porcine small intestinal sucrase which encourages research and development of this glycosidase inhibitor.

Acknowledgements

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