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Towards alpha-glucosidase folding induced by trifluoroethanol: Kinetics and computational prediction

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ABSTRACT

Alpha-glucosidase (EC 3.2.1.20) is an enzyme, which is related with diabetes mellitus type 2 clinically, and is also generally used to convert starch to fermentable sugars in the industry. Therefore, study on this enzyme structures and functions is important. In this study, we investigated structural changes in the alpha-glucosidase during trifluoroethanol (TFE)-induced unfolding. The activity of alpha-glucosidase was significantly inactivated by TFE in a dose-dependent manner. The inactivation was composed of two-phases. TFE inhibited alpha-glucosidase in a parabolic mixed-type reaction ($K_i = 0.72 \pm 0.08$ M). TFE directly induced the unfolding and hydrophobic exposure of alpha-glucosidase. We also simulated the docking between alpha-glucosidase and TFE, as well as molecular dynamics. The computational simulations suggested that several residues, such as ASP68, TYR71, VAL108, HIS111, PHE177, ASP214, THR215, GLU276, HIS348, ASP349, and ARG439, interact with TFE. The molecular dynamics simulation confirmed the binding mechanisms, between the alpha-glucosidase and TFE, and suggested that TFE inhibits the glucose binding site. Our study provides insights into the alpha-glucosidase folding behaviors, and cosolvent binding under a 3D structural simulation.

1. Introduction

Alpha-glucosidase (EC 3.2.1.20) is known to catalyze the hydrolysis of terminal α-(1-4)-linked D-glucose residues from the non-reducing end of alpha-glucoside [1]. Industrially, alpha-glucosidase is used to convert starch to fermentable sugars, which is an important step in alcohol production. This enzyme can also hydrolyze a variety of glucopyranosides, such as sucrose, trehalose, and maltose. Clinically, the inhibitor of this enzyme is associated with treating type 2 diabetes [2–4]: they are oral anti-diabetic drugs, which function by preventing the digestion of carbohydrates and producing sugars. However, few studies have focused on conformational and activity changes during the folding of this enzyme [5–7].

Trifluoroethanol (TFE)-mediated denaturation studies have provided an insight into the enzymes' catalysis, stability, including the forces required to maintain secondary and tertiary structures, conformational flexibility, compactness, and the folding pathways [8–11]. Thus, TFE is generally used to probe structural characteristics of the enzymes. TFE was also used to identify a disease-related protein aggregation [12–14].

Here, we conducted a kinetics study of the alpha-glucosidase, in the presence of TFE denaturant, to expand our understanding of the alpha-glucosidase structure, which integrates with the computational simulations. Kinetic rate constants and other parameters were calculated, and the unfolding pathway for alpha-glucosidase is discussed. Probing and characterization of conformational intermediates, during the alpha-glucosidase folding, is an important goal of enzymatic research. Our TFE-mediated inactivation study of the alpha-glucosidase provides a new insight into an inhibition of this important enzyme.

2. Materials and methods

2.1. Materials

Alpha-glucosidase (*S. cerevisiae*), *p*-nitrophenyl α-D-glucopyranoside, 1-anilino-8-naphthalenesulfonate and 2,2,2-trifluoroethanol were obtained from Sigma–Aldrich (USA). The purchased alpha-glucosidase had a K_m of

Abbreviations: pNPG, p-nitrophenyl α-D-glucopyranoside; pNP, 4-nitrophenol; ANS, 1-anilino-8-naphthalenesulfonate; TFE, 2,2,2-trifluoroethanol.

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0.188 ± 0.002 mM ($V_{max} = 0.239 \pm 0.008$ mM min^{-1}), which was evaluated from the Lineweaver–Burk plot.

2.2. Sample preparation and alpha-glucosidase activity assay

Alpha-glucosidase was incubated in solutions containing different concentrations of TFE (0–12 percent) in 50 mM phosphate buffer (pH 6.8) for 2 h at 25 °C. The alpha-glucosidase activity had been determined at 37 °C by measuring the change of absorbance at 400 nm, which accompanies the hydrolysis of pNPG to generate pNP, according to the previously described method [5,15]. The assay system was composed of 5 mM pNPG substrate and 5 μl of enzyme solution in 1 ml of 50 mM phosphate buffer (pH 6.8), with or without various concentrations of TFE. The alpha-glucosidase activity (v) was monitored with the calibration curve that is constructed with the generation of pNP per min at 37 °C by using a Helios Gamma Spectrophotometer (Thermo Spectronic, UK) at 400 nm.

2.3. Kinetic analysis for the parabolic mixed-type inhibition

For the analysis of a mixed-type inhibition mechanism, the Lineweaver–Burk equation in a double reciprocal form can be written as:

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{[I]}{\alpha K_i}\right) \quad (1)$$

The secondary replot for calculating K_i can be written as:

$$\text{Slope} = \frac{K_m}{V_{max}} + \frac{K_m}{V_{max}K_i}[I] \quad (2)$$

However, K_i cannot be determined directly from the usual equations, due to the parabolic relationship between Slope vs. [I] since the linear relationship is only valid for these equations. Thus, a modified equation was applied with the modification of the previously reported equations [9,16]:

$$\ln(\text{Slope}) = \left(\frac{K_m}{V_{max}}\right)\frac{1}{K_i}[I] + \left(\frac{K_m}{V_{max}}\right), \quad (3)$$

where the reciprocal of the ln(Slope) is replotted versus the corresponding inhibitor. The replot has a slope of (K_m/V_{max}) $1/K_i$ when the plot was linearly plotted. The K_i value can be obtained from the above equations.

2.4. Spectrofluorimetry: intrinsic and ANS-binding fluorescence measurements

According to the previously described method [6], the samples were treated for 2 h in the incubating solutions, which contained different concentrations of TFE at 25 °C. The final enzyme concentration was 3.13 μM. The intrinsic fluorescence spectra were measured with an excitation wavelength of 280 nm and the emission wavelengths ranged from 300 to 400 nm. These data were recorded with an F-2500 fluorescence spectrophotometer (Hitachi, Japan), using a 1 cm path-length cuvette.

The changes in ANS-binding fluorescence intensity were studied by labeling the samples with 40 μM ANS for 40 min, prior to the measurement. An excitation wavelength of 380 nm was used for ANS-binding fluorescence, and the emission wavelengths ranged from 400 to 600 nm. After the enzyme sample was incubated with TFE for 2 h, 20-fold ANS was added to each sample. The final enzyme concentration was 3.13 μM. All reactions and measurements were carried out in 50 mM phosphate buffer (pH 6.8) at 25 °C.

2.5. Homology modeling of the alpha-glucosidase structure and docking simulations

The protein structure for the alpha-glucosidase was developed, using homology modeling because the known structure is not available. HHsearch [17] was used to find template structures, and ten best template sequences were aligned using T-Coffee [18] in its fast mode. Pseudo-Quadratic Restraint Simulated Annealing (PQR-SA) protocol [19] was used to generate the structure from multiple alignment restraints, which was obtained from their structures. From the generated structures, the docking simulation, using AutoDock Vina [20], was performed to find plausible docking sites. The structure of TFE molecule was drawn based on the structure from the PubChem database (CID: 6409).

2.6. Molecular dynamics simulations between alpha-glucosidase and TFE

To examine the interactions between the alpha-glucosidase and TFE, we performed 10 nano-second (ns) molecular dynamics simulations, using CHARMM [21]. The initial structure for the simulation was obtained from the final structure of AutoDock Vina. Using CHARMM-GUI website [22], we had setup the simulation for the alpha-glucosidase and TFE. A generalized Born model, with a simple switch function (GBSW) [23], was used to consider an implicit solvation. The structures were saved every 1 pico-second (ps) for a trajectory analysis. We measured the structural details of the interactions, as a function of time, to ensure which interactions are conversed during the simulations.

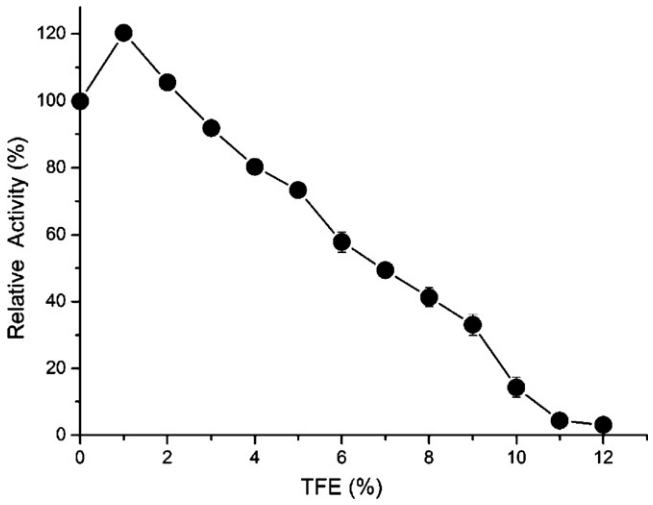

Fig. 1. Effect of TFE on the activity of the alpha-glucosidase. (A) The enzyme was incubated with TFE for 2 h at 25 °C. The activity was then measured with a native control enzyme, by measuring the change of the absorbance at 400 nm at 37 °C. The assay system was composed of 5 mM pNPG substrate, and 5 μl of enzyme solution in 1 ml of 50 mM phosphate buffer (pH 6.8). The final enzyme concentration was 3.13 μM. Data are presented as mean values ($n = 3$).

3. Results

3.1. Inactivation effect of TFE on the activity of alpha-glucosidase

The alpha-glucosidase was slightly activated in a low concentration of TFE (less than 2 percent), and then, as increasing TFE concentration, the activity was gradually inactivated in a dose-dependent manner (Fig. 1). The IC_{50} was evaluated as 7 ± 0.2 percent (0.97 ± 0.028 M). At less than 2 percent TFE, the activity was enhanced to 20 percent, compared to the native state. The activation phenomena by a low concentration of TFE have been observed in some cases: for example, tyrosinase (EC1.14.18.1) was activated by the low concentration TFE of up to10 percent [9], and alkaline phosphatase (EC3.1.3.1) was activated almost up to 60 percent [24], compared to that of the native state. In addition, bovine carbonic anhydrase II (EC 4.2.1.1) is known to be slightly activated, up to 10 percent in the presence of less than 10 percent TFE [25].

When TFE was increased to 12 percent, the enzyme activity was almost abolished, and the TFE worked as a denaturant, as expected. Probably, due to the reason that TFE has a soft electrophilic property, a relatively high concentration was needed to fully inactivate the enzyme. Next, we checked the reversibility of TFE inactivation by evaluating the plots of remaining activity, versus the different concentrations of enzyme at various TFE concentrations (Fig. 2). The results showed straight lines that all passed through the origin, which indicates that the inactivation by TFE was reversible.

3.2. Inactivation kinetics of alpha glucosidase in the presence of TFE

To detect the inactivation kinetics, we performed time-interval measurements and had evaluated the rate constants (Fig. 3). The alpha-glucosidase activity was decreased in a time-dependent manner with the first-order reaction by TFE (Fig. 3A). When TFE concentration was less than 2 percent, the activity was not inactivated, even if it was activated up to 20 percent, compared to that of the native state. It is consistent to the result shown in Fig. 1. At higher than 6 percent of TFE, the activity was, distinctively, inactivated in two ways: in a very short time (less than 30 s), which is not able to monitor properly in our condition, most of the activity of the alpha-glucosidase was quickly abolished, and the rest of the