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Fluorescence and circular dichroism spectroscopy to understand the interactions between cyclodextrins and α-galactosidase from green coffee beans

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ABSTRACT

The potential of fluorescence measurement and circular dichroism spectroscopy (CDSP) to evaluate the interaction between cyclodextrins (CDs) (CD cavity size, concentration, pH, reaction time, and temperature as well as different side chain groups) and α-galactosidase was evaluated. A strong relationship was observed between α-galactosidase fluorescence intensity and CD cavity size, concentration, pH, reaction time, and temperature as well as different side chain groups. Therefore, it can be concluded that fluorescence intensity measurement can be a promising tool to ascertain β-CD-α-galactosidase interactions. CDSP is also an interesting tool to understand β-CD-α-galactosidase interactions as well as to evaluate the stability of α-galactosidase secondary structure. For instance, CDSP results showed that among three different types of CDs studied (α-CD, β-CD and γ-CD), β-CD promoted the most important α-helix transformation into β-sheet, thus reducing α-galactosidase secondary structure stability and showing the highest inhibitory effect on α-galactosidase fluorescence.

1. Introduction

Cyclodextrins (CDs) are cyclic hollow oligosaccharides based on α-(1 − 4)-linked glucopyranoside units, which are able to form inclusion complexes, thus being able to act as molecular containers or carriers (Carmona, Caporaso, Martina, Cravotto, & Mendicuti, 2015). Due to their properties, CDs have been used for different food applications (eg. to develop new products or materials, food analysis, extraction of compounds, masking negative properties of products, modifying enzyme activity, etc.) (Burapatana, Prokop, & Tanner, 2006; Jiang, Guo, & Li, 2010; Li, Jiang, & Wang, 2008)

Among the different CDs complexes, a growing interest has been shown in the complex α-galactopyranosyl-CDs due to its potential use as tool in the delivery of some important healthy compounds (Martina, Puntambekar, Barge, Gallarate, Chirio, & Cravotto, 2009; Yao et al., 2017). α-galactopyranosyl-CDs complex is formed when CDs interact with the enzyme α-galactosidase, which is an exoglycosidase enzyme, being its main role of the enzymolysis of α-galactoside via catalysis of α − 1,6-galactoside bond in the sugar chain ends of polysaccharides, glycolipids and glycoprotein (Anisha, 2017; Tao, Li, & Yao, 2015; Yao et al., 2017).

α-gal, widely exists in animals, plants, and some bacterial broth such as *Bifidobacterium* and *Penicillium* (Anisha, 2017) and has a great importance in the food industry (Cao, Xiong, True, & Xiong, 2016; Kurakake, Okumura, & Morimoto, 2015) and medicine (eg. clearing microvascular endothelial deposits of globotriaosylceramide from the kidneys, heart, and skin in patients with Fabry's disease) (Eng et al., 2001). Therefore, an increased interest in evaluating α-galactosidase activity is shown.

Some previous works have evaluated the impact of CDs on enzymes (Croyle, Roessler, Hsu, Sun, & Amidon, 1998; Kamerke, Pattky, Huhn, & Elling, 2013; Kato et al., 2007; Marcus & Scott, 2001; Robyt, 2005; Yao et al., 2017). It has been observed that the formation of complexes between CDs with special groups of the enzymes has an impact on their activity (eg. inhibition of pullulanase and lipoxygenase) (Li, Wang, Jiao, Xu, & Jin, 2013; Yu & Wang, 2011), as well as reaction rate and equilibrium (Hamilton, Kelly, & Fogarty, 2000; Pinotsis, Leonidas, Chrysina, Oikonomakos, & Mavridis, 2003; Thoma & Koshland, 1960).

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The mechanism underlying this effect is thought to be connected with the host–guest complex formation (Koralewska, Augustyniak, Temeriusz, & Kańska, 2004), change of structure and microenvironment (Yu, Tian, Yang, & Xu, 2011; Yu, Wang, Zhang, & Jin, 2011). Other authors found that the geometric dimension of hydrophobic cavities was crucial for matching between CDs and enzymes (Yao et al., 2017; Yu, Tian, et al., 2011; Yu, Wang, et al., 2011).

Therefore, based on the unique characteristics of CDs and the great importance of CDs-α-galactosidase complex, it is timely to investigate the interaction mechanism between CDs on α-galactosidase. For instance, the impact of CDs on α-galactosidase was evaluated by detecting its catalytic activity using chromogenic assays (Yao et al., 2017). However, absorbance-based assays are usually less sensitive than fluorescence-based assays, and cannot be easily miniaturized.

Some previous studies have shown the ability of CDs, particularly β-CD and its derivatives to modify fluorescence intensity of a variety of fluorescence materials (Carmona et al., 2015; Frankewich, Thimmaiah, & Hinze, 1991; Kinoshita, Linuma, & Tsuji, 1973; Qiu, Huang, & Song, 2012; Zhang, Zhang, & Ma, 2011). Therefore, it could constitute a useful tool to evaluate the interaction between CDs and α-galactosidase, thus facilitating the optimization in the development of α-galactopyranosyl-CDs complexes. However, to the best of our knowledge, at this stage of development it is not clear if CDs are able to change the fluorescence of α-galactosidase.

On the other hand, circular dichroism is an effective tool to investigate the structure of compounds with optically active, organic molecules, that have a chromophore (eg. enzymes), which either possess intrinsic chirality or are placed in chiral environments, and can give rise to circular dichroism signals. Therefore, circular dichroism can be a useful technique to study enzyme structure and function (Abe, Kenmoku, Yamaguch, & Hattori, 2002). For instance, the mechanism of interaction of enzymes with other compounds (eg. CDs) can be revealed through analyzing secondary structure (eg. content of structural features such as α-helix and β-sheet) of the enzyme by analyzing circular dichroism spectrum in the far UV region (240–180 nm), which corresponds to peptide bond absorption (Chen, Yang, & Martinez, 1972; Johnson, 1999; Yao et al., 2017).

In view of this, the main aims of this study are: to evaluate i) the interaction between CDs and α-galactosidase and ii) the modifications of α-galactosidase structure using fluorescence determination and circular dichroism spectroscopy. In order to evaluate systematically the interaction of CDs-α-galactosidase, the impact of CDs cavity size, concentration of β-CD, pH, reaction time, reaction temperature and different side chain groups of β-CD on α-galactosidase from green coffee beans (as model matrix) will be studied.

2. Materials and methods

2.1. Chemicals and reagents

α-galactosidase (EC 3.2.1.22) was extracted from green coffee beans, cyclodextrins (α, β and γ) and *p*-nitrophenyl-α-D-galactopyranoside (PNPG) were purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd. All other reagents were of analytical grade.

2.2. Sample preparation

In this experiment, α-galactosidase and cyclodextrins were reacted in phosphate buffer solution at the designed conditions. Then boric acid-sodium hydroxide buffer solution (pH 9.7) was added to finish the reaction. Finally, the supernatant of reaction liquid was used for the fluorescence measurement (Yao et al., 2017). In order to study the effect of reaction a) time, and b) temperature, c) pH and d) side chain group, on the interaction between β-CD and α-galactosidase, a solution of α-galactosidase (0.2 μM) was added into a solution of β-CD (10 mM), and then incubated a) for 0 min, 30 min, 60 min and 90 min respectively at 25 °C, pH 6.5; for 60 min at 25 °C, 35 °C, 45 °C and 55 °C under pH 6.5, respectively; and c) for 60 min at 25 °C under pH 4.5, 5.5, 6.5 and 7.5 respectively. Finally, a solution of α-galactosidase (0.2 μM) was added into a solution containing different β-CD derivatives (10 mM), and then incubated for 60 min at 25 °C, pH 6.5, to evaluate the impact of side chain group of β-CD on α-galactosidase. All experiments were conducted in triplicate.

2.3. Fluorescence determination

Fluorescence spectra were obtained on a FP-6500 fluorescence spectrometer (Jasco, Japan) with the excitation wavelength of 286 nm and at an emission wavelength of 340 nm, the excitation slit of 5.0 nm. The emission fluorescence in the range between 295 and 500 nm was recorded at the scanning speed of 12,000 nm/min and the emission slit of 5.0 nm (Nørgaard, 1996).

2.4. Circular dichroism spectra

Circular dichroism spectra were scanned at far-UV range (190–250 nm) with J-810 Chiral Detector (Jasco, Japan) in a 0.1 cm quartz cuvette at 25 °C. The concentration of protein for circular dichroism analysis was 1.12 mM. Three scans were averaged to obtain the circular dichroism data, which were expressed in terms of molar ellipticity ([θ]), in degree cm^2/dmol. The estimation of secondary structure was performed using the K_2D method (Yang, Wu, & Martinez, 1986), which is based on the linear regression method of Young's algorithm. For this method, the secondary structure of the protein to be detected is the same to that found for the three kinds of conventional secondary structure of the α-helices, β-folding and random coil of a standard protein. The measured samples were repeated fitting each conventional structure and the percentage of each secondary structure was calculated (Yao et al., 2017).

2.5. Statistical analysis

All statistical analyses were performed using the software SPSS Version 24 (IBM® SPSS® Statistics, USA). Significant differences between the results were calculated by multiple sample comparison of the means (ANOVA) and the LSD test, with a significance level of $p < 0.05$. The error bars presented on the figures correspond to the standard deviations.

3. Results and discussion

3.1. Effect of CDs cavity size on α-galactosidase

To explore the influence of CDs cavity size on α-galactosidase, 0.2 μM α-galactosidase was mixed with different CDs (α-CD, β-CD and γ-CD), and then incubated for 90 min at 35 °C, pH 6.5. As shown in Fig. 1a, a significant ($p < 0.05$) decay in fluorescence intensity was observed when α-galactosidase was mixed with all the CDs, independently of the CDs evaluated, which indicated that each CD could inhibit α-galactosidase. The higher decrease in fluorescence was observed when β-CD was used followed by γ-CD and α-CD. These results are in close agreement to those found by Yao et al. (2017) when they evaluated the interaction between CDs and α-galactosidase by determining the enzymatic activity of α-galactosidase using absorbance-based assays. These authors also observed a stronger inhibitory effect induced by β-CD compared to the other CDs (α-CD and γ-CD).

α-CD, β-CD and γ-CD possess 0.47–0.53, 0.60–0.65 and 0.75–0.83 nm (Szejtli, 1998) cavity internal diameter, respectively. As can be expected, their ability to form complexes with the enzyme differed according to the cavity size. As it is shown in Fig. 1b, the maximum fluorescence intensity of α-galactosidase at 340 nm in the absence of CDs was 206.12, while the maximum fluorescence intensities