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Enhanced fluorescence of starch-fluorescence guest complexes enables evaluation of the encapsulation properties of maize starches

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ABSTRACT

In this study, high-amylose maize starch (HAMS) was mixed with salicylic acid, 1-naphthol, and 2-naphthol, and the physical mixtures were then subjected to a sealed-heating procedure to prepare HAMS-fluorescence guest complexes. The results, obtained from wide angle X-ray scattering and thermogravimetric analysis, showed that all three fluorescence guests were encapsulated in the hydrophobic cavity of HAMS. After screening, salicylic acid was chosen as the most suitable fluorescent probe for evaluating the properties of the starch helix cavity. The formation of a starch-salicylic acid inclusion complex was accompanied by increased fluorescence from the salicylic acid molecules included in the complex. The hydrophobicity of the starch helix cavity and the capability of starch to form inclusion complexes could be evaluated from the increasing intensity of the fluorescence. Accordingly, the capabilities of maize starches to form inclusion complexes followed the order: high-amylose maize starch > normal maize starch > waxy maize starch.

1. Introduction

Starch, which contains amylose and amylopectin, can form inclusion complexes with small molecules. Such inclusion complexes have wide potential applications in the food and pharmaceutical industries, including modification of starch rheological functionality and pasting properties, enhancement of the proportion of resistant starch, reduction of starch retrogradation, and the protection and controlled release of flavor compounds or sensitive bioactives (Obiro, Ray, & Emmambux, 2012). Amylose is well known for its unique ability to form inclusion complexes with hydrophobic guest molecules such as iodine, fatty acids, and aromatic compounds. In the presence of hydrophobic guest molecules, amylose undergoes a conformation change, converting to a single, left-handed helix structure called V-type amylose (Gelders, Vanderstukken, Goesaert, & Delcour, 2004; Obiro et al., 2012). As a result of this conversion, a central channel forms that passes through the axis of the helix, in which the cavity is decisively hydrophobic and the outside surface is highly hydrophilic. The significant hydrophobicity of the central channel is caused by the axial O-1, H-3, H-5, and 6-CH_2 fragment, which occupy the inner surface of the helix, while the hydroxyl groups contribute to the hydrophilicity of the outer surface. This conformation results in a more hydrophobic inner cavity with binding sites for hydrophobic guest molecules (Immel & Lichtenthaler, 2000; Putseys, Lamberts, & Delcour, 2010).

Consecutive turns of V-amylose helices are stabilized by numerous van der Waals forces and hydrogen bonds. The driving force for the formation of inclusion complexes is related to the hydrophobic interactions that occur when guest molecules are transferred from an aqueous to a less polar environment (Fanta, Shogren, & Salch, 1999). The hydrophobicity of the helical cavity is a mandatory prerequisite for the formation of inclusion complexes. Therefore, the degree of hydrophobicity of the helical cavity is the factor that determines the capability of starches from different sources and treatments to complex with guest molecules. Our previous work showed that retrograded starch displays a resistance effect in the presence of iodide ions, which is related to the hydrophobic helical cavity (Tian et al., 2011). Stefan and Frieder ranked the percentage distributions of the surface portions as a function of relative hydrophobicity in a semi-quantitative way. The hydrophobic portion was found to comprise approximately 28% of

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the total surface area per glucose unit in V_H-amylose using MOLCAD software (Immel & Lichtenthaler, 2000). To date, most of the relevant information about these systems has been acquired through theoretical or computational studies, whereas there have been few quantitative experimental studies of hydrophobicity.

In recent years, due to its advantageous properties of high sensitivity and simplicity and convenience of use, fluorescence spectroscopy has been considered one of the most potentially promising techniques widely used in the field of biochemistry and the pharmaceutical industry (Kato & Nakai, 1980; Ullah, Khan, Bilal, Nurjis, & Saleem, 2016). The fluorescent probe method, based on fluorescence variation, is one of the common approaches used to quantify protein hydrophobicity. In general, fluorescent probes, which are hydrophobic, have a low quantum yield of fluorescence in aqueous solution; their quantum yield is related to the polarity of their environment. When probes interact with the hydrophobic region of proteins, the fluorescence intensity increases with the hydrophobicity of medium environment, which is widely used for measuring the hydrophobicity of protein (Alizadeh-Pasdar & Li-Chan, 2000; Pin, Man-Man, & Bin-Sheng, 1996). Stryer reported that the fluorescence quantum yield of 1-anilino-8-naphthalene sulfonate (ANS) was much higher when it combined with apoprotein than when it was in an aqueous environment, resulting in the widespread use of ANS as a fluorescent probe to investigate the hydrophobic regions of proteins (Stryer, 1965). Similarly, when appropriate fluorescent probes complex with starches, the fluorescence intensity can change according to the hydrophobic environment of the starch helical cavity (Poór et al., 2016). Moreover, based on the sodium dodecyl sulfate binding method of determining protein hydrophobicity, the amount of sodium dodecyl sulfate combined with protein is measured to reflect the hydrophobicity of the protein (Kato, Matsuda, Matsudomi, & Kobayashi, 1984). In this study, the amount of fluorescent probe that combined with the starch moiety was indicated by the enhancement of fluorescence intensity, and this reflected the hydrophobicity of the starch. The driving force for the formation of inclusion complexes is the tendency of starch to minimize its interactions with water (Heinemann, Conde-Petit, Nuessli, & Escher, 2001). Therefore, the hydrophobic structure of the starch helix can be reflected by the change in fluorescence intensity upon binding of a fluorescent probe. Thus the capability of starches to complex with guest molecules is reflected by the variations in fluorescence intensity of the fluorescent probe. Therefore, the ability of a given starch to complex with guests can be measured by the fluorescent probe method. To be useful, fluorescent probes must not only fluoresce but must also be incorporated in the helical cavity of starches. Salicylic acid (SA), 1-naphthol (1-NPL), and 2-naphthol (2-NPL), which all contain a phenyl group and are hydrophobic molecules, have previously been investigated as fluorescent probes (Jean-Luc Putaux, Cardoso, Dupeyre, Morin, Nulac, & Hu, 2008; Uchino, Tozuka, Oguchi, & Yamamoto, 2001, 2002). A series of screening experiments is essential to identify the most appropriate fluorescent probe.

In the present work, an appropriate fluorescence probe was selected and the changes in fluorescence intensity upon formation of the starch-fluorescent probe complex were measured. The aims were to provide a method for determining the ability of the starch to form a complex with guest molecules and provide potential evidences for evaluating the hydrophobicity of starch using the enhanced florescence.

2. Materials and methods

2.1. Materials

SA, 1-NPL, and 2-NPL of purity 99% were purchased from Sigma–Aldrich Inc. (Shanghai, P.R. China). They were ground with a mortar before use as fluorescent probe guests. Normal maize starch (NMS) with 26% amylose and high-amylose maize starch with 62% amylose were purchased from STARPRO Starch Co., Ltd. (Hangzhou, China). Waxy maize starch (WMS) with 0.5% amylose was kindly donated by Tianjin Tingfung Starch Development Co., Ltd. (Tianjin, China). All other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Suzhou, China) and were of analytical or spectroscopic grade.

2.2. Preparation of HAMS-fluorescent guest inclusion complexes

The HAMS-fluorescent guest inclusion complexes were prepared by the sealed-heating method as previously described by Tozuka et al. (Nakai, Yamamoto, Oguchi, Yonemochi, & Hanawa, 1991; Tozuka et al., 2006). In brief, HAMS was physically mixed with each fluorescent guest at a weight ratio of 2:1 in a glass vial using a vortex mixer for 5 min. The physical mixtures were sealed in glass ampoules and heated at 120 °C for 1 h to obtain preliminary samples.

2.3. Determination of the inclusion rate of the HAMS-fluorescent guest inclusion complexes

To remove the free fluorescent guest molecules that had not reacted with HAMS, the sealed-heated samples were rapidly washed three times with diethyl ether and dried for 4 h at 25 °C. Then, the pretreated samples were dispersed in anhydrous alcohol and sonicated for 30 min. The resulting solutions were then filtered through a membrane (0.22 μm pore size) prior to analysis. The contents of SA, 1-NPL, or 2-NPL included in the complexes were determined at 296, 297, and 275 nm, respectively, using a UV spectrophotometer (TU-1900, Persee General Instrument Inc., Beijing, China). The encapsulation rate (%) was calculated from the following formula (1):

$$Encapsulation\ rate(\%) = \frac{M_i}{M_t} \times 100 \quad (1)$$

where M_i is the content of the included fluorescent guest in the inclusion complex and M_t is the total content of fluorescent guest molecules in the original physical mixture.

2.4. Characterization of HAMS-fluorescent guest inclusion complexes

2.4.1. Wide angle X-ray diffraction (XRD)

The fluorescence guests, HAMS, physical mixtures, and sealed-heated samples were first equilibrated at 75% relative humidity in desiccators containing saturated sodium chloride for 7 d. XRD experiments were performed on a Bruker D8-Advance XRD instrument (Bruker AXS Inc., Karlsruhe, Germany). All samples were scanned from 4 to 35° (2θ) at a scanning rate of 3°/min with a step size of 0.02° under conditions of 40 kV and 30 mA with nickel-filtered Cu Kα (wavelength 1.5405A) radiation. XRD patterns were analyzed using MDI Jade 5.0 software (Materials Data Inc., California, USA). The degree of crystallinity (%) was defined as the ratio of the area subtracting the area of the amorphous halo from the total area of the diffraction pattern to the total area above the baseline, as follows (2):

$$Degree\ of\ crystallinity\% = \frac{A_c}{A_o} \times 100\% \quad (2)$$

where A_c is the area of crystallized peak and A_o is the overall area