Mechanistic understanding of the relationships between molecular structure and emulsification properties of octenyl succinic anhydride (OSA) modified starches

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ABSTRACT

Octenyl succinic anhydride modified starches (OSA-starches) are widely used as emulsifiers and stabilizers in the food industry. Selection of optimal OSA-starches for a given application would be facilitated with better understanding of the relations between performance and molecular structure, and the mechanistic reasons for observed correlations. Structural parameters, including molecular size distribution, chain-length distribution, degree of branching, degree of substitution (DS) and amount of small molecules such as maltose, were characterized using size-exclusion chromatography (SEC, also termed GPC) and ^{1}H nuclear magnetic resonance (NMR), and were correlated with the functional properties of viscosity, emulsion droplet size and emulsion stability. The observed correlations varied with the methods used to make the OSA-starch, which affects gross structural features. The viscosity of OSA-starch solutions was related to starch molecular size and the relative amounts of small molecules; the DS showed positive correlation with viscosity. Emulsion droplet size is mainly determined by the whole molecular size of the OSA-starch. Emulsion stabilization efficacy is mainly affected by the average chain length. These correlations can be rationalized by basic precepts of the behaviour of polymer chains in solution and the fundamentals of electrosteric stabilization.

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1. Introduction

Octenyl succinic anhydride (OSA) modified starches are emulsifiers with a wide range of applications in the food industry,

including as beverage emulsifiers, hydrophobic ingredients and encapsulating materials (Ackar et al., 2015; Sweedman, Tizzotti, Schäfer, & Gilbert, 2013). The highly branched molecular structure of starch makes it possible for OSA-modified starches to have a wide range of molecular structures and thus a wide range of properties. OSA-starch structural parameters, such as the molecular size distribution, chain-length distribution (CLD), degree of branching (DB) and degree of substitution (DS), can be changed by enzyme or acid degradation of the parent starch and by varying reaction conditions with OSA, thus leading to changes in product viscosity, solubility and emulsion properties. For example, the emulsifying properties of OSA-starch can be improved by β-amylase hydrolysis (Xu, Huang, Fu, & Jane, 2015); β-amylase specifically cleaves (1 → 4)-α glycosidic bonds from the non-reducing ends of starch branches, releasing a maltose for each successful

hydrolysis (Miao, Li, Huang, Jiang, & Zhang, 2015), and the hydrolysis can be stopped by OS groups and branch points ((1 → 6)-α bonds). On the other hand, α-amylase cuts (1 → 4)-α bonds randomly in the interior of starch chains (LeCorre, Vahanian, Dufresne, & Bras, 2012). Acid is usually used to treat starch granules rather than gelatinized starch, hydrolyzing both (1 → 4)-α and (1 → 6)-α bonds (Wang, Blazek, Gilbert, & Copeland, 2012).

OSA-starches with lower molecular weight tend to have higher encapsulation efficiency and capacity (Hategekimana, Masamba, Ma, & Zhong, 2015), while OSA modified starch samples containing larger molecules have better emulsion stability (Dokić, Dokić, Dapčević, & Krstonošić, 2008; Sweedman, Schäer, & Gilbert, 2014). Rigid macromolecules with higher DB can lead to more stable emulsion due to steric effects (Dokić et al., 2008; Sweedman, Schäfer, et al., 2014), but DB itself is less influential than the molecular size and branching chain length for the degraded starches (higher DB) and linear molecules (lower DB) (Scheffler, Huang, Bi, & Yao, 2010). DS also plays an important role in OSA-starches emulsion properties. Emulsifying capacity and emulsion stability are improved with increasing DS. However, the DS values of commercial samples used in these studies were quite similar because of the FDA limitation of the application in food products (3% OSA, DS ~ 0.023).

OSA-starches are branched (electro)-steric stabilizers. The basic precepts of unbranched steric stabilizers are well known (Napper, 1983): the colloidal stability and emulsification properties are largely (but certainly not entirely) controlled by having the water-soluble polymer chains on the surface of the particle or droplet having lowered conformational entropy when two droplets or particles approach. There are additional effects when the stabilizer may be ionized under a particular pH range (an electrosteric stabilizer), but for OSA-starch, it is believed to be predominantly just steric stabilization (Tesch, Gerhards, & Schubert, 2002). However, branched steric stabilizers are poorly understood, at least for highly branched systems such as OSA-modified starches. Although there are abundant investigations on the structural and functional properties of OSA-starches, there is a paucity of results on the mechanistic reasons for observed correlations.

The present paper provides data and mechanistic explanations for such correlation using a range of commercial OSA-modified starches. These commercial OSA-starches were produced by three different hydrolysis methods (α-amylase, β-amylase and HCl), which resulted in significantly different types of molecular microstructures, thereby providing a wide range of structural parameters for exploring structure-property relations. Some of our previous methodologies for understanding these relationships are used in the present paper (Sweedman, Hasjim, Shäfer, & Gilbert, 2014; Sweedman, Schäfer, et al., 2014), but the range of structures used here is much wider and thus the conclusions are more general.

2. Materials and methods

2.1. Materials

OSA modified starches were provided by DSM Nutritional Products Ltd. (Basel, Switzerland), and divided into three groups (A, B and C) according to the degradation processes: α-amylase, β-amylase and HCl, respectively (Supporting Information). The native starches used for modifications by the suppliers are not known, except that they were all waxy maize varieties. However this would not affect making structure-property correlations for the modified starches, which is the objective of the present paper. All modified starches were soluble in water, and thus dispersed in the molecular state, under the conditions used. The molecular structures of these modified starches are all completely characterized in the present

work. Medium-chain triglyceride (MCT) was purchased from Houman Biological Tech. Co., Ltd. (Shanghai, China). Trifluoroacetic acid-d_1 (TFA-d_1) and LiBr were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO)-d_6 was purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Isoamylase from *Pseudomonas* sp. was purchased from Megazyme International, Ltd. (Wicklow, Ireland). DMSO (GR) was purchased from Merck & Co., Inc. (Kenilworth, NJ, USA). Other chemical reagents were analytical grade and used as received.

2.2. Characterization of structural properties

2.2.1. Debranching of OSA-starch samples
Approximately 4−6 mg of each OSA modified starch was debranched using isoamylase following a method described previously (Tran et al., 2011). Specifically, the starch sample was dispersed in 0.9 mL of deionized water, the dispersion heated in a boiling water bath for 15 min to gelatinize, and then cooled to room temperature. Sodium azide solution (5 μL, 0.04 g/mL) and 100 μL sodium acetate buffer (0.1 M, pH 3.5) were added to the starch suspension, followed by addition of 2.5 μL isoamylase solution. The mixture was incubated in a water bath at 37 °C for 3 h, after which it was neutralized to pH 7 with drop-wise addition of 0.1 M NaOH, heated to 80 °C for 1 h, and centrifuged at 4000g for 10 min. The supernatant containing debranched starch was frozen in liquid nitrogen to prevent retrogradation, and freeze-dried overnight. The residues from the debranching procedure, such as denatured enzyme and salts, were not removed from the debranched starch samples, but analysis of these substances alone has shown that they do not interfere with sample peaks (Sweedman, Hasjim, Tizzotti, Schäfer, & Gilbert, 2013).

2.2.2. Size exclusion chromatography (SEC)
The molecular sizes of whole and debranched OSA modified starches were analyzed using SEC after dissolving in dimethyl sulfoxide (DMSO) with 0.5% (w/w) LiBr overnight at 80 °C to yield a final concentration of 2 mg/mL and 4 mg/mL, respectively (Cave, Seabrook, Gidley, & Gilbert, 2009). DMSO/LiBr solution has been proven to completely dissolve starch molecules with minimal degradation (Schmitz, Dona, Castignolles, Gilbert, & Gaborieau, 2009). SEC analyses were performed using an Agilent 1260 Infinity SEC system (Agilent, Santa Clara, CA, USA) equipped with a refractive index detector (RID, Optilab UT-rEX, Wyatt, Santa Barbara, CA, USA). A series of PSS separation columns consisting of GRAM Pre-Column, GRAM 100 and 1000 analytical columns (Polymer Standards Service, Mainz, Germany) placed in an oven at 80 °C were used for both the whole and debranched samples. The volume of each injection was 100 μL, and DMSO containing 0.5% (w/w) LiBr was used as eluent with the flow rate of 0.6 mL/min. The relation between elution volume and the SEC separation parameter, the hydrodynamic volume V_h (or the equivalent hydrodynamic radius R_h) was obtained using a series of pullulan standards (Polymer Standards Service) with peak molecular weight ranging from 342 to 2.35×10^6, which were converted to R_h using the Mark-Houwink equation (Vilaplana & Gilbert, 2010a) with a refractive index increment (dn/dc) value of 0.0717 mL/g, as measured by Michael Krämer and Peter Kilz of PSS, Mainz, Germany. For linear molecules (such as debranched starches), the R_h of debranched starch can be converted to X, the degree of polymerization (DP) through the Mark-Houwink relation. The Mark-Houwink parameters K and α for linear starch dissolved in DMSO/LiBr solution at 80 °C are 0.015 mL/g and 0.743, respectively (Liu, Castro, & Gilbert, 2011). The number distribution, $N(X)$, can be obtained from corresponding weight distribution, $w(\log X)$, using the relation: $N(X) = X^{-2} w (\log X)$ (Clay & Gilbert, 1995; Shortt, 1993). The